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SECTION B

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A COMPARATIVE STUDY OF THE MORPHOLOGY, HISTOLOGY AND PROBABLE FUNCTIONS OF THE PYLORIC CÆCA* IN INDIAN FISHES, TOGETHER WITH A DISCUSSION ON THEIR HOMOLOGY†

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(With 28 Text-Figures, and Plates I-IV)

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I INTRODUCTION

References to literature reveal the fact that while a fairly good account of the morphology of the pyloric cæca has been given in the case of European

* The old term 'pyloric cœs' is being retained in all the pages here for the sake of convenience, though actually these structures arise from the proximal portion of the duodenum.

† Revised and abridged version of the Thesis approved for the Degree of Doctor of Science in the University of Madras.

and other foreign fishes as dealt with in *Handbuch der vergleichenden Anatomie der Wirbeltiere*, Vol III (1937), there is very little systematic account of the comparative anatomy, histology, etc., of these interesting structures in the Indian fishes. Furthermore, as regards our present knowledge of the alimentary tract of fishes in general, very little is known about its histology and that of other structures directly connected with it, and it is for this reason that the author has taken up the present investigation with a view to work out and to present his results in as complete and systematic a manner as possible. A large number of Indian fishes, 119 species in all, included under 50 different families (both fresh-water and marine) have been investigated. In order to minimise the cost of publication and the paper, the original thesis has been considerably boiled down, though maintaining the salient features and continuity, and for the present purpose many text-figures and the photomicrographs have also been cut down.

2 HISTORICAL REVIEW

The structure (and to some extent the physiology) of the pyloric cæca has been described by a number of workers, amongst whom the most notable ones are Rosenthal (1824), Hyrtl (1864), Blanchard (1882), Stirling (1884), Macallum (1886), Bondyoy (1897-1899), Gulland (1898), Johnson (1907), Greene (1912), Kostanecki (1913), Crofts (1925), and Ben Dawes (1930), but the best and most up-to-date works on this subject are those of Jacobshagen, Pernkopf and Lehner described in the *Handbuch d. vergleich Anat d. Wirbelt.*, Vol 3, 1937.

Rosenthal just mentioned the existence of the right and left bunches of the cæca (2-4 in number) in the sword-fish (*Xiphias gladius*) without giving any figure. Hyrtl described a very peculiar and interesting case of the disposition and opening of the "ductus choledochus" (bile-duct) directly into the "Appendix pylorica" in *Fistularia tabacaria* Linn (one cæcum), *Aulostoma chinese* (2 cæca) and *Acanthurus schal* C V. (6 cæca), which has so far not been observed in any other fish—in the last two species of fishes the bile-duct opens into one of the cæca mentioned. Krukenberg, Blanchard, Stirling and Macallum have studied the physiological aspect of the pyloric cæca. A fairly good morphological and histological account has been given by a very few workers, viz., Gulland in the case of the Atlantic salmon (*Salmo salar*), Greene in his description of the king salmon (*Oncorhynchus tschawytscha*), Kostanecki dealing with the morphology and homology of the pyloric cæca as compared with those of other vertebrates; and Ben Dawes on his work on the histology of the alimentary tract of the plaice (*Pleuronectes platessa*). The only valuable work on the anatomy and

histology of the alimentary canal of Indian fishes that I am aware of is the one on *Otolithus ruber* (Bl Schn) by Dharmarajan (*Proc Ind Sci. Cong.*, 1936) and the other on *Therapon quadrilineatus* by Panicker (in MS)—both from the Research Zoological Laboratories at Madras. Sarbahi, D S, has also worked out the Morphology and Histology of the Alimentary Canal of *Labeo rohita* (Hamilton) in detail (*Journ Roy. As Soc of Bengal*, 1940). Mention may be made of Vanajakshi's interesting paper on the Histology of the Digestive Tract of *Saccobranchus fossilis* and *Macrones vittatus* (*Proc. Ind Acad. Sci.*, 1938). On the other hand, Johnson has shown that the pyloric cæca exhibit what might be called an "individuality" of structure and variation existing in one and the same family (*cf* the family Centrarchidae which he has dealt with), while the work of Miss Clifts on an allied topic, *viz.*, on the cæcal gland ("cæcal appendage" or "appendix digitiformis") of Selachian fishes and its homology, etc., is extremely interesting.

3 MATERIAL AND TECHNIQUE

Live fishes were obtained for fixation of material, and the pyloric cæca were thus fixed in fresh condition, which was necessary for a successful study of the finest histological details of these structures. Bouin's fluid and other fixatives such as Zenker's and Mann's fluids, etc., were used throughout the work, the first amongst these gave the best results. For all ordinary morphological work, either the whole fish, if it was small (and in that case with the abdomen slightly opened out), or the pyloric cæca together with some part of the alimentary canal dissected out when the fish was large, was fixed in 6% formalin.

Furthermore, in certain cases, in order to ensure perfect fixation and preservation, the cæca were first of all injected, through the oesophagus, with Bouin's fluid until they were just turgid after suitable ligatures. The cæca and a small length of the associated gut were then removed and preserved in the same fluid for 6–18 hours. After carefully separating the cæca from the surrounding tissues, each, as a rule, if it was a large one, was divided at least into two portions, *viz.*, a proximal and a distal (except in the case of such forms as the Mastacembelidae in which the two cæca are relatively very small and they are very closely situated too). Each portion of the cæcum (and in certain cases a small portion of the intestine also) was imbedded separately in paraffin, and serial sections (both transverse and longitudinal, 6 μ in thickness) were cut.

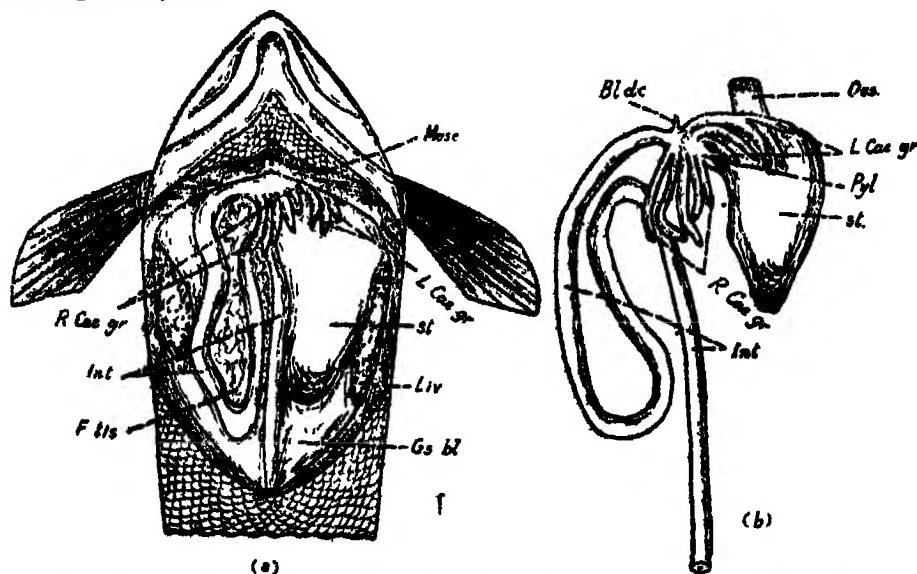
Sections were variously stained, such as, for instance, in Borax carmine counterstained with Picro-indigo-carmine; Mallory's triple, Heidenhain's iron haematoxylin; Mason's iron haematoxylin method coupled with Acid

Fuchsin and Aniline blue; Heidenhain's Azan stain; Pasini's method following treatment with strong Delafield's haematoxylin, and mordanting the sections with 2% Phosphotungstic acid, and then lastly staining with Unna's Wasserblau-Orcein and Giemsa's stain.

Sections were carefully studied with compensating oculars ($\times 10$, $\times 15$ and $\times 7$) in combination with apochromatic objectives (such as 1/12, 40 and 90 with iris diaphragm and numerical aperture 1.25 for oil-immersion purposes). A series of photomicrographs, with both high (including oil-immersion) and low powers, have been taken and then enlarged, and, in addition, many free-hand and camera lucida drawings have also been made and compared. For the detection of cilia in active condition intra-vital staining methods by methylene blue and neutral red were also applied with successful results.

4 MORPHOLOGY OF THE PYLORIC CÆCA IN *Therapon jarbua* (FORSK.) AS A TYPE

The pyloric cæca vary considerably in their number, form, size and arrangement, and constitute a characteristic feature of the intestinal tract of a very large number of families of fishes (both fresh-water and marine), widely distributed in all parts of the world. For the sake of convenience, I shall briefly describe first the condition of the cæca as they exist in a common marine fish, *Therapon jarbua* (Forsk.) (Fam Therapontidae), and shall afterwards present a comparative account of them in other groups of fishes investigated by me.



TEXT-FIG. 1.—(a) Dissection of the viscera from ventral aspect of *Therapon jarbua* showing the cæca in situ ($\times 1$). (b) Alimentary canal of the same fish unravelled showing the disposition of the cæca ($\times 1\frac{1}{2}$).

In *Therapon jarbua* [Text-Fig. 1 (a) and (b)] there are 10 distinct, tubular (finger-like) cæca arranged in two bunches of 5 each, and each cæcum opening independently into the duodenum immediately behind the pylorus. In a fish measuring 9.8 cm. in length, the average lengths of the whole of the alimentary canal (including the œsophagus, stomach and intestine) and the cæca were 12.2 cm. and 9 cm. respectively, and the average length ratio of the cæcum : intestine alone being 1.11 (taking the length of cæcum as a unit). The average breadth of the intestine and that of the cæca in this fish was 4 mm.

The blood-supply¹ of the cæca is as follows —

(a) *Arterial* — There is no separate cæcal artery, but one of the branches of the cœliaco-mesenteric artery distributes blood to the cæca, as is the case in most fishes. All the ten cæca are supplied by ten fine separate twigs arising independently from the gastric branch of the cœliaco-mesenteric

(b) *Venous* — The blood is returned from the right and the left cæcal bunches respectively by two small distinct veins, which ultimately empty themselves independently into the hepatic portal vein.

As regards the nerve-supply² of the cæca the right visceral branch of the vagus divides into four twigs, viz.,

(a) Intestinal innervating the whole length of the intestine

(b) Gastric supplying the stomach, liver, pancreas, etc.

(c) Cæcal—all the cæca

(d) Cardiac—the heart, whereas the left visceral of the vagus possesses all the branches as the right save the cæcal, and its distribution is practically just the same as that of the right.

5. COMPARATIVE ANATOMY OF THE CÆCA IN VARIOUS GROUPS OF INDIAN TELEOSTS

Eduard Pernkopf and Joseph Lehner (1937) give a very comprehensive account of the morphology of the anterior portion of the intestine and the associated structures, especially the "appendices pyloricae" of fishes. The pyloric cæca (pertaining to which they have collected the data from various sources and which they have described) of 234 species, belonging to 78 different families of fishes distributed over Europe, South America and Mexico, are described. A brief summary of their work is given here :—

¹ Emery's Aqueous Carmine was used for injecting the blood-vessels

² 10% of acetic acid (followed by Bouin's fluid) was used to make the nerves prominent in fresh specimens,

(a) The number of "appendices pyloricae" varies from one (in *Fistularia serrata*, *Ammodytes*, etc.) to 909 (in *Merlangus carbonarius*), while a large number of cæca are present in several other teleostean fishes, and also including, e.g., one in *Scymnus lichaia* (a viviparous shark from the Mediterranean and the Atlantic) and two in the Greenland shark (*Lamargus borealis*), but, as a general rule, they are absent in this group of fishes.

(b) The cæca may be quite simple and open independently into the portion of the intestine immediately following the pylorus (or sometimes into the latter), as in *Polyacanthus*, *Amphiprion*, *Rhombus*, etc.

(c) Or, they may be more highly evolved and exist in the form of tufts, each tuft being composed of small cæca varying in number from 3-10; these tufts open into the fore-part of the intestine, as found in the case of *Pelamys*, *Thynnus alalonga*, etc.

(d) Most complicated and highly developed cæca are found in certain Ganoid fishes, viz., *Lepidosteus*, *Acipenser*, etc. In these fishes they form a sort of compact glandular mass which opens into the beginning of the intestine by means of small ducts—four such ducts being present in *Lepidosteus*, and in *Acipenser*, there being no ducts, but one big and two small openings are visible inside the pylorus.

Regarding the fishes investigated by me, the structures in question may all be brought together and placed under 10 main morphological groups. For the sake of clearness, I have illustrated them by a series of diagrams.

The groups are—

GROUP I.—Cæca are quite separate and open independently into the duodenum immediately beyond the pylorus Number of cæca varies.

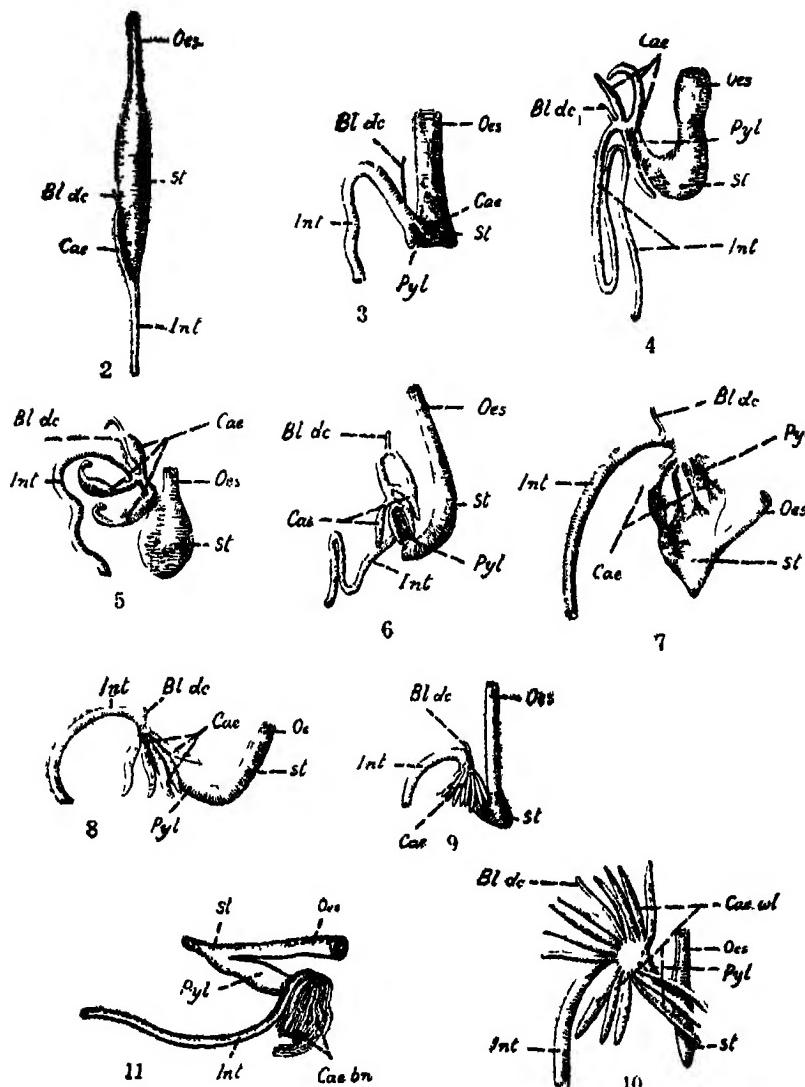
(A) Cæca not in any definite order.

Fam **FISTULARIIDÆ**—*Fistularia villosa* Klunz—the intestine is in a straight line with the stomach. A single cæcum only, arising from the right side (Text-Fig. 2)

Fam **NOTOPTERIDÆ**—*Notopterus notopterus* (Pallas)—2 cæca—one dorsal—and the other ventral in position

Fam **OPHICEPHALIDÆ**—All members of this family possess two finger-like cæca. The following four species have been investigated *Ophicephalus marulius* Ham., *O. punctatus* Bl.; *O. striatus* Bl.: *O. gachua* Ham.

Fam **MUGILIDÆ**—*Mugil cunnesius* C. V.; *M. corsula* Ham.—2 small cæca in each.



Text-Figs. 2-11.—Fig. 2 Portion of the alimentary canal of *Fistularia villosa* showing the disposition of the single cæcum ($\times 1$). Fig. 3 Ditto of *Nandus nandus* showing two small cæca ($\times \frac{1}{2}$). Fig. 4 Alimentary canal of *Pterois russelli* showing three tapering cæca ($\times 1$). Fig. 5 Portion of the alimentary canal of *Abudedefduf sexatilis* showing the disposition of the cæca ($\times 1$). Fig. 6 Ditto of *Pseudorhombus triocellatus* showing four cæca ($\times 1$). Fig. 7 Ditto of *Mugil cephalus* showing five club-shaped cæca ($\times \frac{1}{2}$). Fig. 8 Ditto of *Hepatus nigricans* showing five tapering cæca ($\times 1$). Fig. 9 Ditto of *Acanthocepola abbreviata* showing the disposition of the cæca ($\times 1$). Fig. 10 Ditto of *Thrirocles kammalensis* showing the disposition of fourteen cæca arranged in the form of a whorl ($\times 1$). Fig. 11 Ditto of *Opisthotremus tartoor* showing the disposition of the cæca ($\times 1$).

Fam OSPHRONEMIDÆ — *Osphronemus goramy* Lacép.—2 cæca of unequal lengths, *Colisa fasciatus* Bl Schn—2 cæca of practically equal length.

Fam MASTACEMBELIDÆ.—*Mastacembelus armatus* (Lacép)—Here there are 2, very small, blunt, finger-shaped cæca which are attached to the duodenum immediately behind the pylorus. the cæca open into the former

Exactly similar state of affairs exists in two other members of this family viz., *M. punctatus* (Ham) and *Rhinchobdella aculeata* (Bl.)

Fam NANDIDÆ *Nandus nardus* (Ham)—2 very small stumpy cæca. (Text-Fig. 3)

Fam LEIOPHANTIDÆ.—*Leiognathus rufonius* (Ham) and *L. equulus* (Forsk)—Each having 3 cæca

Fam GERRIDÆ.—*Gerres filamentosus* C V. and *G. abbreviatus* Blkr.—3 cæca in each

Fam PHIPPIDÆ.—*Drepane punctata* (L.)—3 cæca

Fam ANABANTIDÆ.—*Anabas testudineus* (Bl.)—3 cæca

Fam. SCORPAENIDÆ.—*Pterois russelli* Bennett—3 cæca (Text-Fig. 4)

Fam POMACENTRIDÆ.—*Abudefduf sexatilis* (L.)—3 small conical cæca (Text-Fig. 5), and *Abudefduf cochinensis* (Day)—3 small cæca

(B) Cæca arranged in the form of a whorl

Fam BOTRIDÆ.—*Bothus pantherinus* (Rupp)—4 club-shaped cæca. *Pseudorhombus triocellatus* (Bl Schn)—4 cæca (Text-Fig. 6).

Fam PLATACIDÆ.—*Platax pinnatus* (L.)—4 small cæca

Fam PSETTODIDÆ.—*Psettodes erumei* (Bl Schn)—11 cæca, each opening independently into the duodenum

Fam TFUTHIDIDÆ.—*Sigarus oramin* (Bl Schn.), *S. java* (L.), *Tenthis marmorata* (Q G)—Each having 5 cæca

Fam MUGILIDÆ.—*Mugil cephalus* L.—5 cæca (Text-Fig. 7); *M. carinatus* C V.; *M. persica* H B; *M. speigleri* Blkr have 5 cæca, all arranged in a 'whorl'

Fam LUTIANIDÆ.—*Lutjanus fulviflamma* (Forsk)—5 club-shaped cæca. 5 cæca are also found in other members of the genus *Lutjanus*, such as *L. kasmira* (Forsk), *L. argentimaculatus* (Forsk); *L. sebae* (C. V.); *L. rivulatus* (C V) and *L. lunulatus* (Mungo Park)

Fam SPARIDÆ.—*Nemipterus tulu* (C V)—There are 9 tapering cæca,

Fam. KYPHOSIDÆ.—*Kyphosus cinerascens* (Forsk)—5 club-shaped cæca.

Fam ACANTHURIDÆ.—*Hepatus nigricans* (L.)—5 tapering cæca (Text-Fig 8)

Fam SCORPÆNIDÆ.—*Parascorpaena bleekeri* (Day)—5 cæca, *Pterois volitans* (L)—15 cæca

Fam SCIENIDÆ.—*Sciæna coiter* (Ham)—6 cæca; *Pama pama* (Ham)—10 cæca

Fam POLYNEMIDÆ.—*Polydactylus heptadactylus* (C V)—6 cæca

Fam ECHIPTERIDÆ.—*Leptecheneis naucrates* (L)—8 cæca

Fam CEPOLIDÆ.—*Acanthocepola abbreviata* (C V)—9 cæca (Text-Fig 9)

Fam KURTIDÆ.—*Kurtus indicus* Bl—9 cæca

Fam URANOSCOPIDÆ.—*Uranoscopus guttatus* C. V—10 small tapering cæca

Fam CHÆTODONTIDÆ.—*Hemochus acuminatus* (L)—6 cæca *Chætodon xanthocephalus* Bennett—10 cæca *Holacanthus xanthuris* Bennett—11 cæca *Pomacanthus annularis* Bl—more than 30 cæca

Fam SCATOPHAGIDÆ.—*Scatophagus argus* (Bl)—20 small cæca

Fam ENGRAULIDÆ.—*Thriissocles kannalensis* (Blkr)—14 cæca arranged in the form of a 'whorl' (Text-Fig 10) *Anchoviella tri* (Blkr)—13-14 cæca; *Engraulis malabaricus* C V—20-25 cæca, *E. pirava* (Ham)—50-55 cæca

Fam CLUPPIDÆ.—*Opisthonterus tartour* (C V)—15-18 cæci (Text-Fig 11), *Pellona ditchoa* C V—30-35 cæca

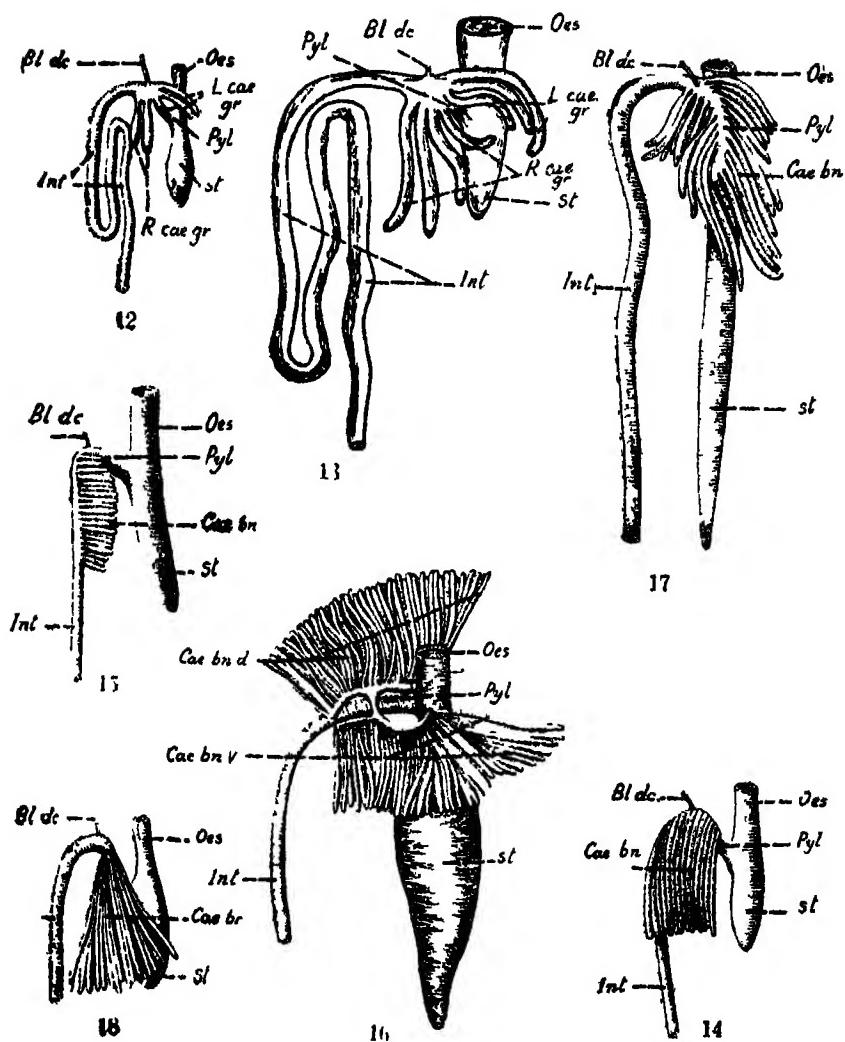
(C) Cæca arranged in the form of two definite groups—right and left—each cæcum opening independently at the beginning of the duodenum

Fam. SILLAGINIDÆ.—*Sillago sihama* (Forsk)—4 club-shaped cæca arranged in two groups of two in each group (Text-Fig. 12)

Fam. SPARIDÆ.—*Sparus sarba* (Forsk)—4 cæca—2 are found pointing towards the left side and two towards the right *Nemipterus japonicus* (Bl)—8 cæca arranged in two groups of 4 in each group

Fam. SERRANIDÆ.—*Lates calcarifer* (Bl.)—5 club-shaped cæca—2 being in the left and 3 in the right group.

Fam. MENIDÆ:—*Lactarius lactarius* (Bl. Schn)—6 cæca in all, arranged in two groups—the right group consists of 2 cæca and the left of 4 cæca.



Text-Figs 12-18—Fig 12 Portion of the alimentary canal of *Sillago sihama* showing the disposition of the ceca ($\times 1$) Fig 13 Alimentary canal of *Cephalopholis argus* showing the disposition of the ceca ($\times \frac{1}{2}$) Fig. 14 Portion of the alimentary canal of *Alepis djedaba* showing the linear arrangement of the ceca ($\times 1$) Fig 15 Ditto of *Harpodon nehereus* showing the disposition of the ceca ($\times \frac{1}{2}$) Fig 16 Ditto of *Sphyrna jello* showing the peculiar arrangement of the ceca on an H-shaped duct ($\times 1$) Fig 17 Ditto of *Trichiurus senegalensis* showing the linear arrangement of the ceca on two sides of the duodenum, ($\times \frac{1}{2}$) Fig. 18 Ditto of *Polydactylus sexfilis* showing the arrangement of the ceca in the form of a 'mop' ($\times 1$).

Fam PLATYCEPHALIDÆ—*Grammoplites scaber* (L)—the cæca are 6-7 in number and arranged in groups of 3 and 3-4 on the ventral side of the stomach, *Platycephalus indicus* (L)—9 cæca arranged in two bunches of 4 and 5 cæca respectively

Fam SCIÆRIDÆ—*Otolithus ruber* (Bl Schn)—5 cæca in two groups of 3 and 2 on both sides of the duodenum, *Johnius dussumieri* (C V)—6 cæca in two groups of 3 cæca in each, *Otolithus brunneus* (Day)—7 cæca arranged in groups of 4 and 3 on the right and left sides respectively *Pseudosciaena axillaris* (C V)—8 cæca arranged in two groups of 5 on the right side and 3 on the left side, *Paneus* (Bl)—12 arranged in two groups of 6 cæca each

Fam THERAPONIDÆ—*Therapon jarbua* (Forsk)—10 tubular cæca arranged in two groups of 5 in each, *T theraps* (C V)—8 cæca; *T puta* (C V)—8 cæca—in each case the cæca are arranged in two groups of 4 in each group

Fam SFRRANIDÆ—*Promicrops lanceolatus* (Bl.)—8 cæca arranged in two groups of 5 on the right side and 3 on the left side, *Cephalopholis bennack* (Bl)—9 cæca—5 on the right side and 4 on the left; *C argus* (Bl Schn)—7 cæca—4 in the right group and 3 in the left (Text-Fig 13).

Fam POMADASIDÆ—*Pomadasys olivaceus* (Day)—6 cæca arranged in two groups, 3 in each, *P hasta* (Bl)—8 cæca, *P furcatus* (Bl Schn.)—6 cæca, *P maculata* (Bl)—5 cæca arranged in two groups; *Scolopsis vosmæri* (Bl)—7 cæca arranged in two groups of 4 on the right side and 3 on the left

Fam MULLIDÆ—*Upeneus vittatus* (Forsk)—9 cæca arranged in two groups—right group of 4, and the left of 5 cæca

GROUP II—*Cæca arranged in a linear series and each cæcum opening independently at the beginning of the duodenum*

Fam SCORPAENIDÆ—*Minous monodactylus* (Bl Schn)—4 cæca arranged in a linear series on the duodenum immediately behind the pylorus.

Fam. CARANGIDÆ—*Alepis djedaba* (Forsk)—15 long tubular cæca arranged in a linear series on the duodenum—they do not form any tuft (Text-Fig 14).

Fam. HARPODONTIDÆ:—*Harpodon nehereus* (Ham.)—19 cæca arranged in a single linear series on the duodenum (Text-Fig 15).

Fam SPHYRÆNIDÆ—*Sphyræna jello* (C V)—A very interesting case is presented by this species: (Cf Text-Fig. 16)—here there are about 700

tabular cæca in all arranged in two linear groups (anterior and posterior), each group consisting of about 50 cæca. These cæca do not open directly into the duodenum, but open respectively into the long limb of an H-shaped duct which lies more or less parallel to the proximal portion of the duodenum—finally the median connecting duct (of the horizontally disposed "H") opens into the duodenum as usual by a single orifice. The stomach is quite large and fusiform, so that the whole thing presents the remarkable appearance of a palm-tree. *S. obtusata* (C V)—here there are 26 cæca in all—17 in the anterior row and 9 in the posterior row, which open by means of an H-shaped duct as in the previous species.

Fam. TRICHIURIDÆ—*Trichiurus sayala* (C V) here there are 32-34 elongated, cylindrical type of cæca arranged on both sides of the proximal region of the duodenum giving the appearance of a bunch of much elongated structures—they open independently into the duodenum (Text-Fig. 17).

GROUP III—Cæca bunched together and arranged in the form of a 'brush' or 'mop' and opening into the duodenum

Fam. POLYNEMIDÆ—*Polydactylus sexfilis* (C. V)—here there are 15 long, tubular and tapering cæca arranged in the form of a 'mop' so to say—the blind-end of all the cæca pointing backwards (Text-Fig. 18).

GROUP IV—Arborescent type of cæca.

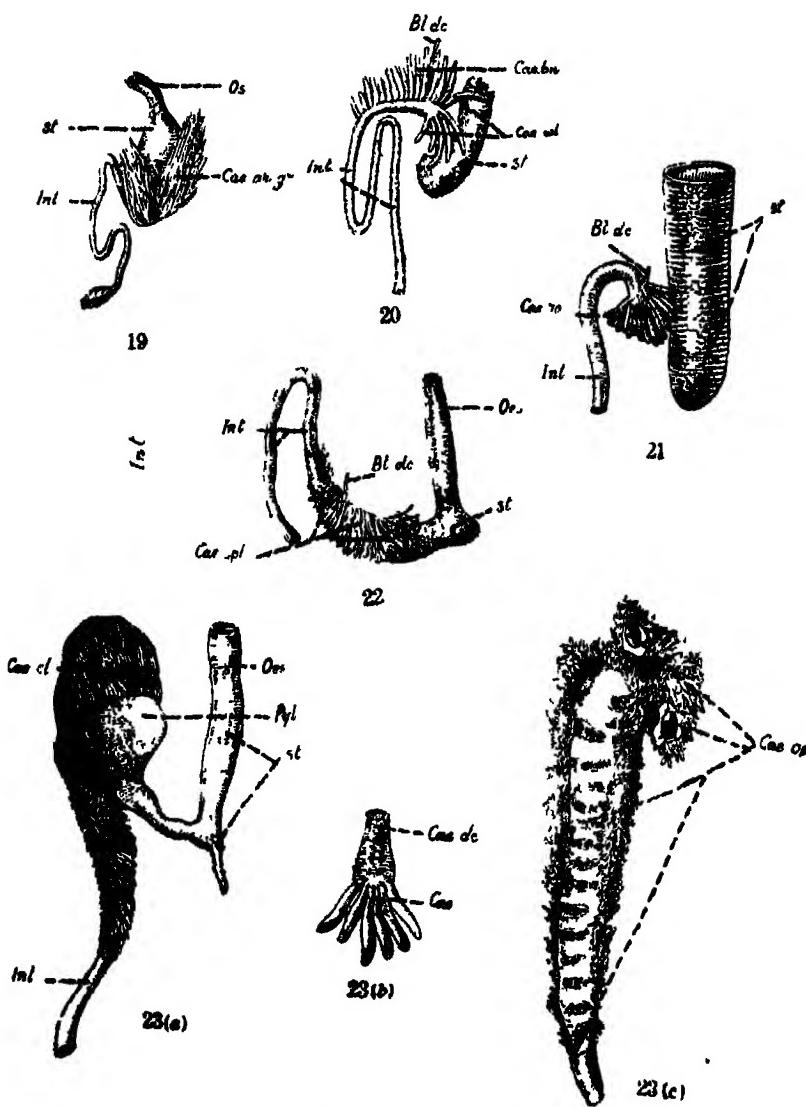
Fam. MENIDÆ—*Mene maculata* (Bl. Schn.)—numerous, slender, long tufts of cæca—each tuft consisting of 3-4 small cæcal diverticula (Text-Fig. 19).

GROUP V—Cæca arranged partly in whorl and partly in linear series:

Fam. HOLOCENTRIDÆ—*Holocentrum ruber* (Foisk)—cæca 25 in number, and out of these 9 are arranged in a 'whorl' and 16 in a linear series (Text-Fig. 20); *Myripristis melanostictus* (Blkr)—17-18 cæca, arrangement more or less the same as above.

GROUP VI—Cæca arranged and grouped together in the form of a rosette (= "Cæcal Rosette")

Fam. CARANGIDÆ—*Caranx malabaricus* (Bl. Schn.)—Cæca numerous and grouped together in one place in the form of a rosette—this consisting of several tufts of cæca, and each tuft being composed of 3-4 small cæcal diverticula (Text-Fig. 21); *C. leptolepis* (C V.), *Alepis kalla* (C. V.) and *A. mate* (C V.), have got the same sort of cæca and the same arrangement as in *C. malabaricus*.



Text-Figs 19-23 Fig. 19 Alimentary canal of *Mene maculata* showing the arborescent arrangement of the casca ($\times 1$) Fig. 20 Ditto of *Holocentrum ruber* showing the disposition of the casca ($\times \frac{1}{2}$). Fig. 21 Ditto of *Caranx malabaricus* showing the arrangement of the casca in the form of a 'rosette' ($\times 1$). Fig. 22. Ditto of *Scyrris indica* showing the arrangement of the casca in a spiral form ($\times 2$) Fig. 23 (a) Portion of the alimentary canal of *Hilma thuka* showing the peculiar disposition of the casca ($\times 1$) Fig. 23 (b). A single cascum of the same fish showing the arrangement of the cascal diverticula ($\times 4$). Fig. 23 (c) Portion of the alimentary canal of the same fish dissected out to show the cascal openings ($\times 1$)

GROUP VII —Cæca arranged in the form of a Spiral (= “ Cæcal Spiral ”):

Fam CARANGIDÆ.—*Scyrus indica* (Rupp)—numerous cæca are arranged in a spiral manner round the commencement of the duodenum immediately behind the pylorus (Text-Fig. 22).

GROUP VIII.—Numerous small cæca arranged in the form of bunches, clusters or tufts and studded thickly over the duodenum :

Fam CLUPEIDÆ.—In the majority of the members of this family the clustering of the pyloric cæca is very characteristic—the highest concentration being reached in the case of a very favourite fish of Bengal and U.P., viz., “ Hilsa ” or “ Ilish ”, *Hilsa ilisha* (Ham.)

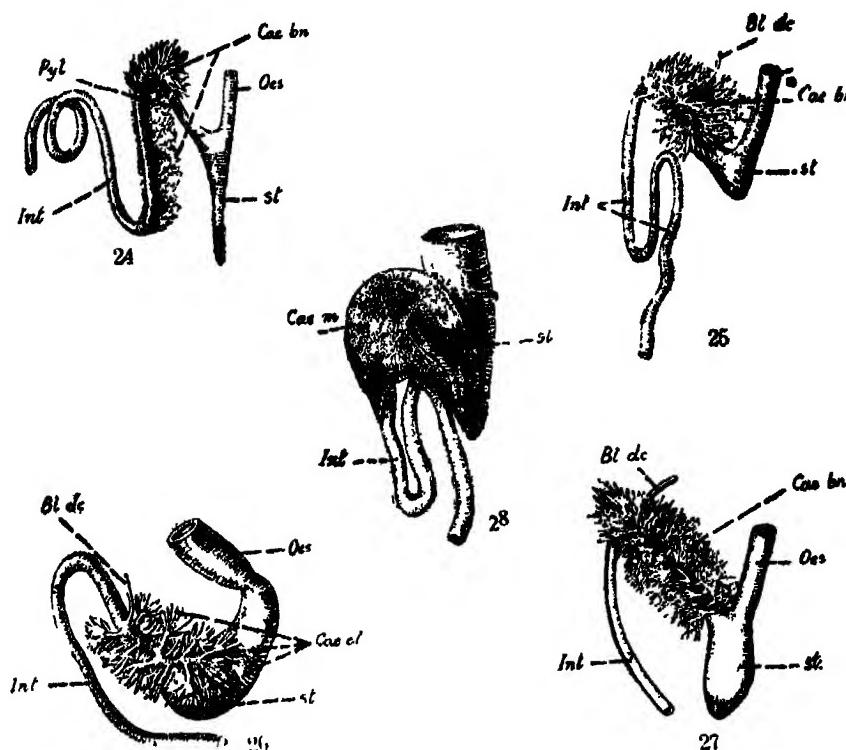
In this family the stomach [Text-Fig. 23 (a)] is more or less V-shaped, its proximal part being tubular, and in this group the pylorus (*pyl.*) is strongly developed due to the preponderance of its muscular walls and assumes a more or less globular appearance.

In *H. ilisha* the cæca exist in the form of *clusters* or *tufts* and are extensively distributed. They are studded immediately behind the pylorus and extend to some distance over the duodenum—such a condition is absent in any other group of fishes so far investigated. Towards the proximal region of the duodenum the cæcal tufts are slightly longer and are massed together to form a sort of “ cap ” lying over the “ head ” of the pylorus—each tuft being of a complex nature and in its turn being composed of 10–12 very small cæcal diverticula; whereas as we trace backwards, the cæcal diverticula gradually get reduced in number till towards the hinder region of the cæcal mass each tuft is composed of 6–8 cæcal diverticula [Text-Fig. 23 (b)]. Just at the beginning of the duodenum most of the cæcal tufts unite together and open by two large ducts, whereas over the rest of the duodenum there are a series of openings (*cæ. op.*) along its inner walls [Text-Fig. 23 (c)], usually a linear group of three, as shown by slitting open of the duodenal wall.

Clupea (Harengula) fimbriata (C. V.) and *C. (Harengula) atricaudata* Gunthr.—the structure and arrangement of the cæca more or less very similar to those of *H. ilisha*.

C. (Harengula) longiceps (C. V.)—here two kinds of cæcal diverticula are found—smaller ones in the form of tufts and elongated ones in the form of small tubes—both being situated over the commencement of the duodenum.

Fam DOROSOMIDÆ.—*Anodontostoma chacunda* (Ham.)—Cæca more or less of the same pattern as in *Hilsa ilisha*, but each tuft is composed of a fewer number of small cæcal diverticula.



Text-Figs 24-28.—Fig. 24 Portion of the alimentary canal of *Elops machnata* showing peculiar disposition of the caeca ($\times \frac{1}{2}$). Fig. 25 Ditto of *Chorinemus tala* showing the disposition of the caeca ($\times \frac{1}{2}$). Fig. 26 Ditto of *Pampus argenteus* showing the disposition of the caeca ($\times \frac{1}{2}$). Fig. 27 Ditto of *Scomber microlepidotus* showing the disposition of the caeca ($\times \frac{1}{2}$). Fig. 28 Ditto of *Rachycentron canadum* showing the compact gland-like mass of the caeca ($\times \frac{1}{2}$).

Fam. ELOPIDÆ.—*Elops machnata* (Forsk.)—There are numerous small caeca arranged in bunches or tufts over the commencement of the duodenum—each tuft being composed of 2-3 very small caecal diverticula, and each tuft opens separately into the duodenum (Text-Fig. 24).

Fam. SERRANIDÆ.—*Serranus tauvina* (Forsk.)—caeca are numerous and are arranged in the form of tufts—each tuft being composed of 3 very small caecal diverticula, like the fingers of a glove.

Fam. CARANGIDÆ.—*Chorinemus tala* (C. V.) Day—here a large number of caecal tufts are grouped together round the commencement of the duodenum, opening into it independently, and each tuft is composed of 2-3 caecal diverticula (Text-Fig. 25).

GROUP IX —Caeca with more complicated and secondarily branched tufts

Fam. STROMATEIDÆ.—*Pampus argenteus* (Euphrason)—here the cæca exist in the form of large number of small tufts which are secondarily branched, each of which consists of 4-6 small cæcal diverticula. The tufts do not open directly into the duodenum, but all of them communicate together through a single duct which runs parallel to the pylorus and ultimately opens at the commencement of the duodenum (Text-Fig 26)

Fam CARANGIDÆ.—*Parastromateus niger* (Bl)—the cæca are dendritic in form as in the above-mentioned species

Fam SCOMBRIDÆ.—*Scomberomorus guttatus* (Bl Schn)—here also the tufts and their diverticula are arranged on the same plan as in *Pampus argenteus*—each tuft being composed of 5-7 small cæcal diverticula, and the common duct runs parallel to the stomach and joins the duodenum, forming more or less a right angle with the latter, *Scomber microlepidotus* Rupp—here the cæca are considerably branched and then secondarily re-branched, and are massed together over the proximal region of the duodenum (Text-Fig 27)

GROUP X—Compact gland-like Cæcal Mass.

Fam RACHYCENTRIDÆ.—*Rachycentron canadum* (L)—(Text-Fig 28)—here the cæca are extremely numerous and arranged in tufts most highly developed, and represent the highest stage of development and modification, each tuft having 3-5 small cæcal diverticula, and the whole mass is enclosed inside a tough connective-tissue sheath, thus presenting, more or less, a gland-like appearance comparable to that already described in the case of Sturgeon (*Acipenser*), Whiting (*Gadus merlangus*) and Tunny (*Thunnus thynnus*)

6 COMPARATIVE STUDY OF THE HISTOLOGY OF THE PYLORIC CÆCA

In fact, practically all the characteristic features of the post-pyloric intestine are repeated in the structure of the pyloric cæca, which may thus be regarded as an integral part of the alimentary tract in this region—in other words the pyloric cæca, in essential respect, exhibit the same histological structure as the anterior part of the intestine (i.e., the duodenum), and are in open communication with this part of the alimentary canal

As a typical case, first of all, I shall describe briefly the histological condition of the pyloric cæca in a marine fish, *Lutjanus lunulatus* (Mungo Park)—(Pl. I, Fig. 1)

In a transverse section through the proximal part of the cæcum the following principal layers are seen from without inwards (i.e., from outside towards the lumen).—

(a) Serosa (*Ser*)—single layer, extremely thin with attenuated squamous epithelial cells resting on a subserous connective tissue base

(b) Longitudinal muscular layer (*long musc*) having longitudinally disposed fibres—this varies in different regions of the cæcum in different fishes

(c) Circular muscular layer (*Circ. musc*)—a relatively thicker sheet of transversely disposed fibres This also behaves in the same way as (b)

(d) Submucosa (*Sub muco*)—a layer of more or less loose areolar connective-tissue in which are embedded numerous, very fine blood-vessels and nerve-fibres It may be differentiated into the following three parts from without inwards —

(i) Stratum granulosum (*St gran*)—characteristic layer of small cells

(ii) Stratum compactum (*St comp*)—homogeneous layer of fibrous connective tissue

(iii) Tunica propria (*Tun pro*) extends from the epithelial layer of the mucosa up to the stratum compactum = usual areolar connective tissue meshwork with numerous blood-vessels and certain special type of small cells

(e) Mucosa (*Muco*)—It is of variable thickness and presents the most interesting modifications in different fishes It is considerably folded showing long finger-like structures (in sections), thus presenting the false appearance of villi, while the mucosal depressions in between them give a false impression of the crypts of Lieberkühn, and for the sake of mere convenience of description these villi-like structures or processes will be designated here as the "cæcal villi" (*cæ vil*)

It may be remembered here that although the mucous folds, to some extent, simulate villi and crypts of higher animals, but these structures do not exist in any fish is shown by the absence of the lacteals and the granular cells such as those of Paneth

The whole of the mucous epithelium is composed of a very large number of tall, slender columnar cells interspersed with numerous goblet-cells (*Gob c*) and a few wandering leucocytes as well Along the inner edge the mucous epithelium is usually fringed with a row of very fine cilia—the cilia are commonly met with amongst many Indian fishes that I have studied, i.e., both in this species as well as in others (and amongst many others, for example, cilia are very distinctly seen in *Hepatus nigricans* (Pl 1, Fig 2, *cil*), and *Pterois russelli* (Pl. I, Fig 3, *cil*).

As is seen in Pl I, Fig 1, so many of the "cæcal villi" are cut in a transverse section. The average diameter of the lumen of the cæcum of *Lutjanus fulvus* is 21.73 μ , and the average length and breadth of the "cæcal villi" are 5.13 μ and 2.26 μ respectively.

It is extremely interesting to note that the histological character (chiefly the folds of the mucous membrane, their proliferations or branching, the union or fusion of these proliferations with one another, and the thickness of the muscular walls, etc) of the pyloric cæca represents a good deal of variations along its different lengths.

Comparing the sections through the *proximal* region of the cæcum (as the one described—*vide* Pl I, Fig 1) with those of the *distal* region (Pl I, Fig 4) the following differences are at once noticeable in the latter—

- (i) The mucous folds greatly proliferate
- (ii) They unite to form a large number of what may be called inter-communicating passages or channels (*Ch*) inside the cæcal lumen (*cæ. lum*)

Again, when the above section (*i.e.*, of the *distal* region) is compared with the one passing *nearer the tip* of the cæcum of the same fish (Pl I, Fig 5), then there is still greater proliferation of the "cæcal villi" seen, and greater number of inter-communicating channels are also visible.

When, however, the section passes through *the very tip* of the cæcum of the same fish, one finds very much changed conditions as follows—

- (i) The muscle-layers are very greatly thickened
- (ii) The cæcal lumen is greatly filled up and divided up into a large number of inter-communicating channels, though it is never completely obliterated

N.B.—The presence of cilia has been recorded in connection with the structure of the pyloric cæca in some fishes described by a very few authors, *viz.*, Edinger (1876) and Tinkler (1884). Macallum (1886) observed a ciliated epithelium in the œsophagus of *Amia*, *Lepidosteus* and *Acipenser*, and even in the stomach of the first-named fish. Sullivan (1907) describes a similar epithelium in the œsophagus of the Elasmobranchs. It appears that, as a rule, such a ciliated epithelial layer does not occur in the pyloric cæca in the majority of the Teleostei, as neither Greene (1912) nor Stirling (1884) observed it in the King-salmon and herring respectively. Gulland (1898) unfortunately omits a consideration of the pyloric cæca in the salmon, but in the plaice it is seen that cilia are absent from every part of the tract.

Nor did Pilliet (1894) observe a ciliated epithelium in any of the Pleuronectidae he studied (Ben Dawes, p 266)

Ishida (1935), however, describes a well-developed band of ciliated epithelium in the intestine of a Blennid fish, *Salarias enosimae*. Furthermore, he has quoted 11 species of teleostean fishes (in addition to a few species of the Cyclostomata, Selachii and Ganoidae), certain portions of the alimentary canal of which as described by different authors, possess cilia, and, amongst these, it is very interesting to note that there are seven species of the former group (*i.e.* Teleostei, *viz.*, *Rhomhus aculeatus*, *Perca fluviatilis*, *Uranoscopus scaber*, *Dactylopsetra volitans*, *Naucrates ductor*, *Scorpaena* sp and *Mullus barbatus*) in which the openings of the pyloric cæca are endowed with cilia

The structure of the cæcum of *Lutjanus fulvus* as compared with that of the intestine of the same fish is best understood by reference to the photomicrograph (Pl I, Fig 6). In the intestine, as one would easily see the "intestinal villi" are of simpler character without any proliferations of its mucous folds, and the goblet-cells are very few in number too. In all other respects its structure is practically just the same as that of the cæcum

A similar state of affairs as seen just now in the case of *Lutjanus* is also best depicted in the case of another marine fish, *Abudefduf cochinensis* (Fam Pomacentridæ)—cf the four photomicrographs (Pl II, Figs 7, 8, 9 and 10). The inter-communicating channels (Fig 10, Ch.) are so very distinct in this fish

Here, in this fish the average diameter of the lumen of the cæcum is 17.53 μ , and the average length and breadth of the "cæcal villi" are 9.75 μ and 2.29 μ respectively

The general condition of the histology of the cæcum of *Therapon jarbua* is shown in two photomicrographs—one through the proximal region (Pl II, Fig 11) and the other through the distal region (Pl II, Fig 12) of the same fish. In the distal region the tremendous amount of branching or proliferations of the mucous folds or the "cæcal villi" and their fusion resulting in the formation of so many inter-communicating channels (Ch) may be noted. As a rule it may be mentioned that the inter-communicating channels do exist towards the distal end of the cæcum in the majority of the Indian teleosts, but in certain exceptional cases, as for example in *Siganus java* the "cæcal villi" remain quite simple and stumpy, and do not proliferate.

Sometimes the inter-communicating channels inside the cæcal lumen are formed towards the bases of the "cæcal villi" due to their proliferation in this region, or, in other words, towards the periphery of the cæcal lumen,

as for instance, in *Hepatus nigricans* (Pl III, Figs 14 and 15), *Holocentrum ruher* (Pl III, Figs. 16 and 17), *Pterois russelli* (Pl III, Fig 18 and Pl IV, Fig 19) and *Serranus tauvina* (Pl IV, Fig 24)

As a rule, also, the "cæcal villi" are highly vascular, thus possibly increasing the absorptive surface. The vascular strands (or capillaries) are distinctly seen in sections of *Leptecheneis naufrates* (Pl IV, Fig 20, *vas cap*), *Abudedefduf cochinensis* (Pl II, Fig 7, *vas cap*) and several others.

Pl IV, Fig 23 is a photomicrograph through the *proximal* region of a single tuft of *Serranus tauvina* (having three very small cæcal diverticula), showing the club-shaped and slightly branched nature of the "cæcal villi"—in this photograph the condition of things in the median diverticulum is fully represented, whereas the two lateral diverticula are only partially represented. Fig 24 in the same plate is a section through the *distal* region of one of the cæcal diverticula of the same fish showing the interdigitation of the "cæcal villi" and the formation of the inter-communicating channels.

In the conclusion of this chapter it may be stated in a summarised form that—

1 The *proximal* part of the pyloric cæca is very similar in histological characters to the structure of the duodenum from the proximal end of which they arise.

2 But in the *distal* part of the cæca the structure becomes quite complicated there is great infolding of the mucous folds, ultimately leading to the interdigitation of the "cæcal villi" and their fusion to form a sort of network inside the cæcal lumen, thus increasing their absorptive surface, but in some cases they remain fairly simple.

3 After a very critical histological examination and study no specially differentiated digestive glands (comparable to the gastric glands, or the intestinal glands analogous to glands of Lieberkühn in higher vertebrates) have been noticed in connection with the pyloric cæca. In all probability the function of the digestive glands in these structures is performed by their ordinary lining epithelium, as in the case of the mucous epithelium of Cyclostomata, Diplopoda and *Amphioxus*.

7 PHYSIOLOGY OF THE PYLORIC CÆCA

The physiology of these very interesting structures, presenting such a diversity of form and occurring in so many different groups of fishes distributed in all kinds of waters of the world, is yet very little known. Various theories have been put forward as to the probable functions of these organs.

by a very few workers, and it may be worthwhile, just for the sake of comparison, to give a brief summary of their work here —

(a) The earliest opinion, and for a time then generally accepted, was that they act collectively as a pancreas in fish in the absence of the latter organ

(b) In 1860, Mordecai (*Bull U.S. Fish Comm.*) advanced the view that these organs collect and store up fluid nutritious matter as a sort of reserve, which, during the passage of the fish to its spawning ground, is absorbed and utilised to repair the wasting processes of the body in the absence of ordinary food

(c) According to Edinger (*op. cit.*), the cæca serve to suck up the liquid and digested food matter as it escapes from the stomach and absorb it

(d) Wiedersheim (*Lehrbuch der Vergl. Anat.*, p. 565) also adopts this view, and tries to show that the development of the pyloric appendages stands in inverse relation to that of the spiral valve.

(e) Krukenberg (*Kuhne's Untersuchungen*, Bd. II, 1882) found in the cæca of the common Sturgeon (*Acipenser sturio*) evidence of the presence of diastase, pepsin and trypsin

(f) According to Stirling's view (*Journ. Anat. and Physiol.*, Vol. XVIII, p. 426), these organs subserve different functions in different fishes, being absorptive in some, and in others glandular, and secreting trypsin in some cases in fairly appreciable quantities

(g) Blanchard (1882) found that the extracts of the pyloric cæca in various fishes affect the transformation of boiled starch into grape sugar, and that when boiled white of egg or fibrin is added to the extract, whether this is alkaline, acid, or neutral in its reaction, peptones are formed. He believes that a tryptic ferment alone is present to accomplish the latter result

(h) Macallum (1886) working on the pyloric cæca of *Acipenser* found that Lipase, pepsin and trypsin were present but no diastase was found. He suggests some secretory functions for these structures also

(i) Greene (1913) mentions in the case of the king salmon (*Oncorhynchus tschawytscha*) that the chief function of the pyloric cæca is fat absorption

(j) Ben Dawes (1930) thinks that the cæca in *Pleuronectes platessa* are reservoirs of food material and are probably both secretory and absorptive

(k) Chesley (1934) working on the "Concentration of enzymes in certain marine fishes" has arrived at the conclusion that the pyloric cæca are more active in fishes in which the pancreas is diffused and that the cæca seem

partially to supplant it in enzyme secretion, but where the pancreas is well developed the cæca have a minor rôle. And furthermore, he says that lipase is least in quantity in fishes having a fatty metabolism.

Physiological Experiments —

For the presence or absence of the digestive enzymes in the pyloric cæca, I have carried out a series of physiological experiments and biochemical tests, and a summary of the latest methods, technique and procedure adopted, the tests performed and all the observations made is given below —

1 *Preparation of the material* — Half dozen large-sized specimens of a common fresh-water carnivorous fish, *Ophicephalus striatus* Bl (Fam Ophicephalidae), were dissected after pithing, and the contents of the pyloric cæca were ground with 5 c.c. of Glycerol in a mortar with some thoroughly washed and sterilized sand. It was then centrifuged, and the fluid was drained into two tubes marked (A) and (B) in equal quantities. Tube (A) was put in a water-bath and kept in boiling water for 30 minutes to kill all the enzymes and was kept as control. Tube (B) contained the test substance.

2 *Demonstration of the presence of Diastase* — When tested with very dilute solution of iodine, the test substance gave positive results for diastase by decolorising iodine, but the reaction was negative for the control.

3 *Demonstration of the presence of Maltase* — When a drop of test and control was put on a litmus paper after keeping both the tubes overnight in an incubator at 40° C and then impinged with a jet of steam, the test substance assumed a brick-red colour, but there was no change on the control. This shows the presence of Maltase.

4 *Demonstration of the presence of Lipase* — A drop of olive oil was added to the test and control and then left overnight in the incubator at 40° C. Formation of an orange coloured ring at the top shows the presence of lipase which converted the oil in emulsion partly into fatty acid. There was no ring formation in the control.

5 *Fibrin-carmine test for the detection of Pepsin* — Fibrin-carmine test was applied and it was found that owing to the hydrolysis of fibrin by the action of pepsin the test substance turned pink, but there was no change in the control.

6 *Detection of Trypsin (after Roaf's modification) by Congo Red test* — Congo Red-stained fibrin was put in two tubes containing fluid from (A) and (B) and kept at 40° C inside an incubator. Control showed practically no colour, whereas the test substance was deeply stained red as a result of the action by the active proteolytic enzyme, i.e., Trypsin.

7. *Detection of Bile*—Although bile was actually seen inside the lumen of the pyloric cæca during dissections, yet for the sake of confirmation, qualitative biochemical test was also applied. This is best demonstrated by means of the well-known standard Gmelin's test which gave positive results

8. *Determination of the pH of the cæcal contents*—The pH of the contents of the cæca was found to be 7.5 in *Ophicephalus* and 7.2 in *Goramy*, and is, therefore, very slightly on the alkaline side

All the above-mentioned series of biochemical tests were also carried out and repeated on the contents of the pyloric cæca of another fish of the same family, viz. *Ophicephalus punctatus* Bl., which gave exactly similar results as in the case of *O. striatus* Bl., and, furthermore, these experiments and tests were done on the contents of the pyloric cæca of a *herbivorous fish*, *Osphronemus goramy* Lacép also, with the same results

Here, below I give in a comparative tabular form the results of the various experiments performed by a few workers (as quoted previously) and also my own results, side by side, showing the presence of the different enzymes in the lumen of the cæca.—

Krukenberg	Stirling	Blanchard	Macallum	Rahimullah
Diastase, Pepsin and Trypsin present and absorption of the digested food	Different functions in different fishes absorptive in some, and in others glandular and secreting Trypsin in some cases	Diastase and Tryptic Enzymes present, which act in acid or alkaline medium	Lipase, Pepsin and Trypsin present, but no Diastase Suggests some secretory functions also	Diastase, Maltase, Lipase, Pepsin, Trypsin and Bile present pH 7.5 (<i>Ophicephalus</i>) 7.2 (<i>Goramy</i>), and hence very slightly alkaline

From the above results it is quite evident that the pyloric cæca perform digestive function to some extent, supplementing the stomach, but practically in all other respects they are very similar in action to that of the intestine, in correlation with their very close resemblance to the latter in histological details

Having detected the presence of certain enzymes inside the lumen of the pyloric cæca, the next question that at once struck me was (a) whether the cæca are secretory at all or not and (b) if so, which of these enzymes are actually secreted by them, and (c) which come from other sources?

For this purpose half a dozen very large-sized live specimens of *Ophicephalus striatus* Bl and *Osphronemus goramy* Lacép were pithed, stomach, pyloric cæca and intestine dissected out, split open and their inside

was thoroughly washed with running distilled water so as to get rid of all the contents, and then scrapings of the mucous membrane were taken by means of a sharp piece of glass and the samples prepared separately as before for *control* and *test* substances. Each of these samples was subjected to typical biochemical tests for the presence of the different kinds of enzymes as mentioned so clearly in the foregoing pages, and the results are shown in the table below —

	Organs	Pepsin	Diastase	Maltase	Lipase	Trypsin
<i>Ophicephalus</i> <i>strumatus</i> Bl. (Carnivorous)	Stomach	+	-	-	-	-
	Intestine	-	-	+	-	+
	Cæca	-	-	-	+	+
<i>Goramby</i> Lacq. (Herbivorous)	Stomach	+	-	-	-	-
	Intestine	-	-	+	-	+
	Cæca	-	+	-	-	+

This shows clearly that in *Ophicephalus*, Lipase and Trypsin are secreted by the cæca, and other enzymes, viz., Pepsin by the stomach, Maltase and Trypsin by the intestine, and some of these are also supplemented by the pancreas, while in "Goramby," Diastase and Trypsin are secreted by the cæca, and the rest come from the stomach, intestine and the pancreas. Here, it may be noted that in the herbivorous fish (*Goramby*), Diastase is secreted in place of Lipase (secreted by the cæca of *Ophicephalus*, a carnivorous fish), because the former is vegetarian and hence requires more of diastase to digest the excess of starchy food.

It is also very interesting to note that the location, formation and distribution of such digestive enzymes as pepsin and trypsin (which are, as a rule, mainly associated with the stomach and pancreas respectively in the higher vertebrates) are very variable in different species of fishes, and not nearly so strictly localised as in the former group of animals. So far from peptic digestion being limited to the stomach, it may take place even in the pharynx, stomach and in the intestine of *Ammocetes* and in some Elasmobranchs (e.g., *Scyllium*), and in certain Teleosts, such as the Pike, Fel and Carp, the peptic region in these types extending from the stomach up to some distance along the intestine, while trypsin is widely distributed in fishes and has been obtained from the mucous membrane of the stomach, intestine and pyloric cæca as well as the pancreas (*C N H Series, Fishes*, p. 271).

I am, therefore, led to conclude that the pyloric cæca serve as accessory reservoirs of semi-digested food and, to some extent, perform the function of digestion also; absorption of digested food possibly takes place in the same way as in the case of the intestine, secretion of *lipase* and *trypsin* takes place in the case of the carnivorous fish viz., *Ophicephalus* and that of *diaspis* and *trypsin* in the herbivorous fish, *Gordmy*

8. DISCUSSION

Cæcal structures other than the pyloric cæca are found in some fishes and other animals too, but they are not homologous with the pyloric cæca of teleostean fishes, and their origin also is so very different indeed, being always found at the junction of the small and large intestine. Therefore, a very brief summary of the cæcal structures is given here —

(1) Functional cæca at the junction of the large and small intestine are usually known to be present in all the Selachian fishes and probably absent in Teleostomi.

(2) In Batrachia, Reptilia and Aves the cæcal diverticula are of a variable nature, but they are always developed in the same relative position, that is to say, at the junction of the large intestine (chiefly colon) and the small intestine.

(3) Functions of cæcal structures in various other vertebrates

(a) In Birds—a digestive and a problematical blood function

(b) In Reptiles—solely digestive, but a double function (viz., digestive and blood-forming) may possibly exist both in Amphibians and Reptiles, because of the presence of a large amount of lymphoid tissue in the cæca

(4) Cæcal gland of Selachian fishes is probably homologous with the cæca in Batrachia, Reptilia and Aves (proved for the first time by Howes by the blood-circulation as well as the presence of lymphoid tissue in both the types of structures)

(5) All other writers consider that the cæcal gland is so very different from the "vermiform appendix" and the "cæca" of other vertebrates

The Pyloric Cæca:-

Here I will just deal with the nature and significance of the pyloric cæca, in a summarised form, as they exist in various groups of fishes in general —

(1) The pyloric cæca are absent in Cyclostomata, Diplopoda, and practically in all Molluscs, but there is a considerable variation as to the number,

form and structure of these cæca in various members of the Teleostomi, some of which have already been described in the foregoing pages of this paper, whilst several have been previously dealt with by other authors

(2) For instance, amongst the 'Ganoids' the pyloric cæca are absent in the Bow-fin (*Amaia*). In "Bichir" (*Polypterus*) there is a single, small cæcum, whereas in other members of this group, viz., Sturgeon (*Acipenser*), Spoonbill (*Polyodon*), and Gar-Pike (*Lepidosteus*) the cæca are very well developed and usually occur in large numbers.

(3) Again, in certain groups of Teleosts the cæca are entirely absent, as for example, in the Cat-fishes (Siluridae) Carps (Cyprinidae) Pike (Pisces) Toothed-carps (Cyprinodontidae), Wrasses (Labridae) and Plectognathi.

After comparison of the various lengths of the alimentary canals and the cæca in relation to the volume of the abdominal space in some fishes, I have obtained certain very interesting results which are as follows:

Measurements were taken of two fishes of practically the same length, viz., one (*Scomber microlepidotus* Rüpp.) having numerous cæca and the other, viz., one of the common Carps (*Labeo calbasu* Ham.) having no cæca, both these fishes have mixed diet. To get a comparative idea, the measurements are given below —

	<i>Scomber microlepidotus</i>	<i>Labeo calbasu</i>
1 Length of the fish	18.5 cm	18.4 cm.
2 Length of the intestine	17.2 "	350.5 "
3 Volume of the abdominal cavity	43.9 c cm	462.0 c cm

This shows that in the case of *Scomber* the volume of the abdominal cavity is decidedly much less in comparison to that of the *Labeo*, and it may be possible that the pyloric cæca might have arisen in the former fish in order to economise the space for the adaptation of the alimentary canal in the smaller space, whereby the length of the intestine has become much less and the pyloric cæca have arisen evidently to compensate for and supplement the functions of the alimentary canal.

Another batch of flat-fishes (Pleuronectids) was taken to elucidate this point as well. In *Synaptura cornuta* (Kaup) in which there are no cæca the intestine was 11.2 cm. in length and the volume of the abdominal cavity was 110.0 c cm. whilst in the case of *Psettidess erumei* (Bl. Schn.), having 11 cæca of practically the same length, the intestine was 3.8 cm. long and

the volume of the abdominal cavity was 21 $\frac{3}{4}$ c.cm. This example also clearly points to the same conclusion as arrived at in the previous case.

(4) Further, six specimens of (*Ophicephalus striatus* Bl.), a fish with two cæca and six of another (*Therapon jarhu* Forsk.) with ten cæca were also taken for comparison, and the following measurements were recorded—

	S No	Fish length	Total length of the cæca	Length of the intestine	Ratio Cæca Intestine
<i>Ophicephalus striatus</i> Bl.	1	20.7	6.5	16.7	1 2.6
	2	22.4	9.1	20.9	1 2.2
	3	27.8	10.9	21.8	1 2.0
	4	28.8	13.7	23.3	1 1.7
	5	31.2	17.0	24.5	1 1.4
	6	38.8	23.0	27.6	1 1.2
<i>Therapon jarhu</i> (Forsk.)	1	5.0	2.5	5.1	1 2.4
	2	6.0	3.3	5.6	1 1.7
	3	7.5	3.8	5.8	1 1.5
	4	8.5	5.6	6.2	1 1.1
	5	11.1	9.7	9.5	1 .98
	6	14.6	11.2	10.8	1 .96

In both the examples it would be apparent that as the fish grows in size the total length of the pyloric cæca becomes greater in comparison to the length of the intestine.

(5) In some other groups of bony fishes, including the European and other foreign as well as the Indian types (both marine and freshwater) the cæca are very variable in number—they may be extremely numerous and extensive (*i.e.*, at least more than 50 in number) *e.g.*, in Salmon (*Salmo*), Whiting (*Garulus merlangus*), Mackerel (*Scomber scombrus*) and most species of Clupeidæ. Amongst Indian fishes the cæca present a beautiful series of most interesting structural modifications, morphological differentiation and numerical gradations as shown in the list given on previous pages, *say*, from One cæcum (*e.g.*, in *Fistularia villosa*—Fistularidæ), Two cæca in Ophicephalidæ, Notopteridæ, Mastacembelidæ certain Mugilidæ, Nandidæ, etc., Three cæca in Leognathidæ, Ephippidæ, Scorpænidæ, Pomacentridæ, etc.:

Four in Bothidae, Platacidæ, Sillaginidæ, etc., Five to Six Centropomidae, Kyphosidae, Lutjanidae, Sciaenidae, etc.; More than 10-25 in Engraulidae (e.g., *Opisthoteretus taroor*, *Engraulis malabaricus*, etc.), Harpodontidae, Holocentridæ, Trichiuridae, etc., to the Very Numerous, highly complicated and extensive types, usually existing in the form of dense tufts or whorl of cæcal outgrowths as found in Sphyrænidæ (about 100 cæca), certain Engraulidae (e.g., *Engraulis purava* = 50-55 cæca), Clupeidae (*Hilsa ilisha* = Several Hundreds), Dorosomidae (*Anodontostoma chacunda*), Serranidae (e.g., *Fpinephelus undulatus* and *Serranus tauvina*), certain Carangidae (*Caranx malabaricus* = cæca in the form of a "Rosette"), Menidae (*Mene maculata*—cæca disposed in Arborescent form), Stromateidae and Scombridæ (cæca arranged in Dendritic form as in *Pampus argenteus* and *Scomberomorus guttatus* respectively).

It may appear at first sight from the above description that the pyloric cæca might be of great taxonomic value in the specific determination of fishes and may be applied for systematic investigations, but, so far as my observations are concerned, and taking other works also into consideration, I have come to the conclusion that they may help to a very little extent in this direction, as generally they are so very different even in the members of one and the same family that no such unusual importance could be given to them.

The number and disposition of the cæca is not a constant factor, and varies even amongst the different genera and species of a single family, as will be perfectly clear from some of the species of the family Therapontidae and the genus *Therapon* —

<i>Therapon puta</i>	.	8	cæca
<i>T. jarbua</i>	..	10	"
<i>T. theraps</i>	.	8	"
<i>T. quadrilineatus</i>		18	"
<i>T. argenteus</i>	.	11	"

(6) It is also very interesting to note that in certain extreme cases, e.g., Sturgeon, Whiting, Tunny (*Thunnus thynnus*) and *Rachycentron canadum* where the cæca are not only numerous, but most of them (or in some cases all of them) are also united together by means of connective tissue to form a compact, gland-like mass communicating with the intestine, either by a single, wide duct (as in Sturgeon), or by several small orifices as in several other examples. Such a condition of the pyloric cæca would apparently lead to the assumption that probably they have some sort of secretory function, supplementing the actions of the digestive glands, such as the liver and the pancreas. It may be said that although no true definite glands are present

inside them, yet the general mucous epithelium of these organs is partly secretory in function. At any rate, however, one might say that such cæca must be of some important use in the fish in which they occur and have assumed such compact, gland like character.

(7) It is not yet quite certain if a particular type of diet has any definite influence on the relative size and structure of these cæca. This does not look very probable, as the cæca are found both in purely vegetarian fishes like *Goram* and also in purely carnivorous fishes like *Ophicephalus*, whereas they are totally absent in some other fishes having the same sort of diet.

(8) Regarding the physiology of the pyloric cæca, the biochemical tests performed by me show the presence of certain important enzymes such as diastase, maltase, lipase, pepsin and trypsin (in addition to bile) inside the cæcal lumen of *Ophicephalus striatus* Bl. and *Osphronemus goramy* Lacép., and, furthermore, it has also been definitely established by similar tests that out of so many enzymes present, only two, viz., lipase and trypsin, are being actually secreted by the cæcal mucous membrane in *Ophicephalus* and diastase and trypsin in *Goram*. The flow of bile inside the cæca has actually been observed in several fishes as has also been confirmed by biochemical tests in *Ophicephalus striatus* Bl and *Osphronemus gordmy* Lacep. It thus follows that some enzymes like maltase and pepsin (in addition, bile) are produced in certain other quarters, i.e., stomach, intestine, pancreas and liver as in higher vertebrates, and then they find their way into the pyloric cæca, whereas a few other enzymes such as diastase, lipase and trypsin are actually, at least to some extent, secreted by the pyloric cæca themselves. This should not be taken as a general rule, unless, of course, a very large number of fishes have been biochemically investigated from this point of view, but, for the present, the actual facts as observed by me have simply been stated here.

The flow and presence of the bile into the cæca, as actually observed and confirmed by biochemical tests, also suggests strongly its rôle upon the action of lipase. Bile emulsifies the fat and breaks it up into innumerable fine droplets which thus offer a large surface for the better and more efficient action of lipase.

(9) No phylogenetic importance can possibly be attached to these cæca, because they are present in greater numbers in the scale of evolution in the fishes much lower down, such as Bothidae, Serranidae etc.; but in *Fistularia villosa* (Fistulariidae) there is only one cæcum. Moreover there is no particular relationship or gradual gradation of the cæca, as we pass from one lower family to the next higher one.

M. Rahimullah (Quraishi)

9 SUMMARY AND GENERAL CONCLUSIONS

(1) In all, pyloric cæca ("Appendices pyloricae") of 119 species belonging to 50 different families of fishes from Indian waters (both fresh-water and marine), have been investigated for this paper

(2) Morphologically the pyloric cæca present the most interesting and a beautiful series of structural modifications and gradations, which may be classed under 10 main morphological heads or groups starting from the simplest condition of the cæcum (as represented by *Fistularia villosa* Klunz—Fam. *Fistulariidae* = only one cæcum) and then passing through many intermediate and more complex groups until the culminating point is reached and represented by the most complex type of the pyloric cæca in the two families, viz., *Stromateidae* (e.g., *Pampus argenteus* Euphrasen, and *Parastromateus niger* Bl.) and *Rachycentridæ* (*Rachycentron canadum* L.)—in this last-named species the cæca assume the structure, more or less of a compact gland-like mass enclosed in a definite sheath comparable to that existing in Sturgeon (*Acipenser*), Whiting (*Gadus merlangus*) and Tunny (*Thunnus thynnus*)

(3) Histologically, in general the pyloric cæca are very similar to the intestine (= duodenum) of which they are outgrowths, and originate by a process of evagination—they however differ from the latter only in details—

(a) No true villi and crypts of Lieberkühn are present in fishes like those of higher vertebrates—the mucosa is thrown into a considerable number of folds which have been described as the "cæcal villi" in this paper

(b) Presence of a variable number of goblet cells and several wandering leucocytes in the mucous epithelium

(c) The mucous epithelium of the cæca of some Indian fishes bears a row of very fine cilia detection of which has been possible due to the study of very thin sections (1 μ , 6 μ under an oil-immersion magnification varying from 1200-1500 circ) and also due to the intra-vital staining methods

So far the presence of cilia in the pyloric cæca of a few other teleostean fishes has been recorded by Ishida (cf *Annotat Zool Japonen*, Vol 15, No 2, 1935, pp 158-60)

(d) The "cæcal villi" present a very different arrangement and complexity of structure in the different regions of the cæcum—in the *proximal* region of the cæcum, they are long, cylindrical, finger-like, highly vascular structures, whereas towards the *distal* region there are a lot of proliferations, interdigitation and fusion of these "cæcal villi", resulting in the formation of several intercommunicating channels, great obliteration of the cæcal lumen

and a sort of more or less "spongy-tissue" (derived from the fused mucosal folds) inside it. at the very tip, however, the muscular walls are considerably thickened and the inter-communicating channels are highly pronounced

(e) The highly vascular nature of the "cæcal villi" is evidently meant for the increase of the absorptive surface and thus supplements the function of the "intestinal villi" in the fishes which possess cæca

(4) The pyloric cæca ("Appendices pyloricae") are outgrowths arising from the proximal region of the duodenum (*and not from pylorus*), and hence the name pyloric cæca is a misnomer they should better be called *intestinal cæca*. They are not homologous with and bear no relationship whatsoever with any other cæcal appendages, such as the rectal gland (or the cæcal gland or "appendix digitiformis"), the "vermiform appendix" and "cæca" of other vertebrates

(5) The probable functions that might be assigned to the pyloric cæca are as follows —

(a) Possibly act as a storage of reserve of semi-digested food-material

(b) Probably absorb the digested food-matter like the intestine

(c) Are said to be mainly concerned with fat-absorption

(d) Partly act as digestive organs—presence of certain enzymes, such as diastase, maltase, lipase, pepsin and trypsin and also that of the bile, which affect the digestion of carbohydrates and proteids, and thus help and supplement the digestive processes of other juices poured into the alimentary canal

(e) The presence of diastase, lipase and trypsin actually inside the epithelial lining of the mucosa of the pyloric cæca clearly points towards their being partly secretory in function also

(6) It is not yet certain what exact correlation exists between diet and the significance of these cæca in fishes, *i.e.*, to say, what exact relationship exists between the nature and complexity of the pyloric cæca on one hand and the herbivorous, carnivorous or omnivorous habit of fishes on the other. But I think that the pyloric cæca have arisen in those fishes where the abdominal space has to be economised owing to the influence of some environmental factors

(7) The study of a large number of fishes proves that the pyloric cæca have practically no taxonomic importance in the classification of fishes; and, if there is any, it is very little indeed

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12 EXPLANATION OF PHOTOMICROGRAPHS

PLATE I

Photo-

- micrograph 1 TS of proximal part of the cæcum of *Lutjanus lunulatus* showing the arrangement of "cæcal villi" and presence of goblet cells
- . 2 TS of the proximal part of the cæcum of *Hepatus nigricans* showing very fine fringe of cilia along the mucous epithelium
- . 3 TS near the tip of the cæcum of *Pterois russelli* showing interdigititation and formation of inter-communicating channels and large number of fine rows of cilia
- . 4 TS of the distal part of the cæcum of *Lutjanus lunulatus* showing proliferation of the "cæcal villi"
- . 5 TS towards the tip of the cæcum of *L. lunulatus* showing the fusion of "cæcal villi" to form channels
- . 6 TS of the intestine of *L. lunulatus* showing the general condition of the mucosal folds as compared with those of the cæcum

PLATE II

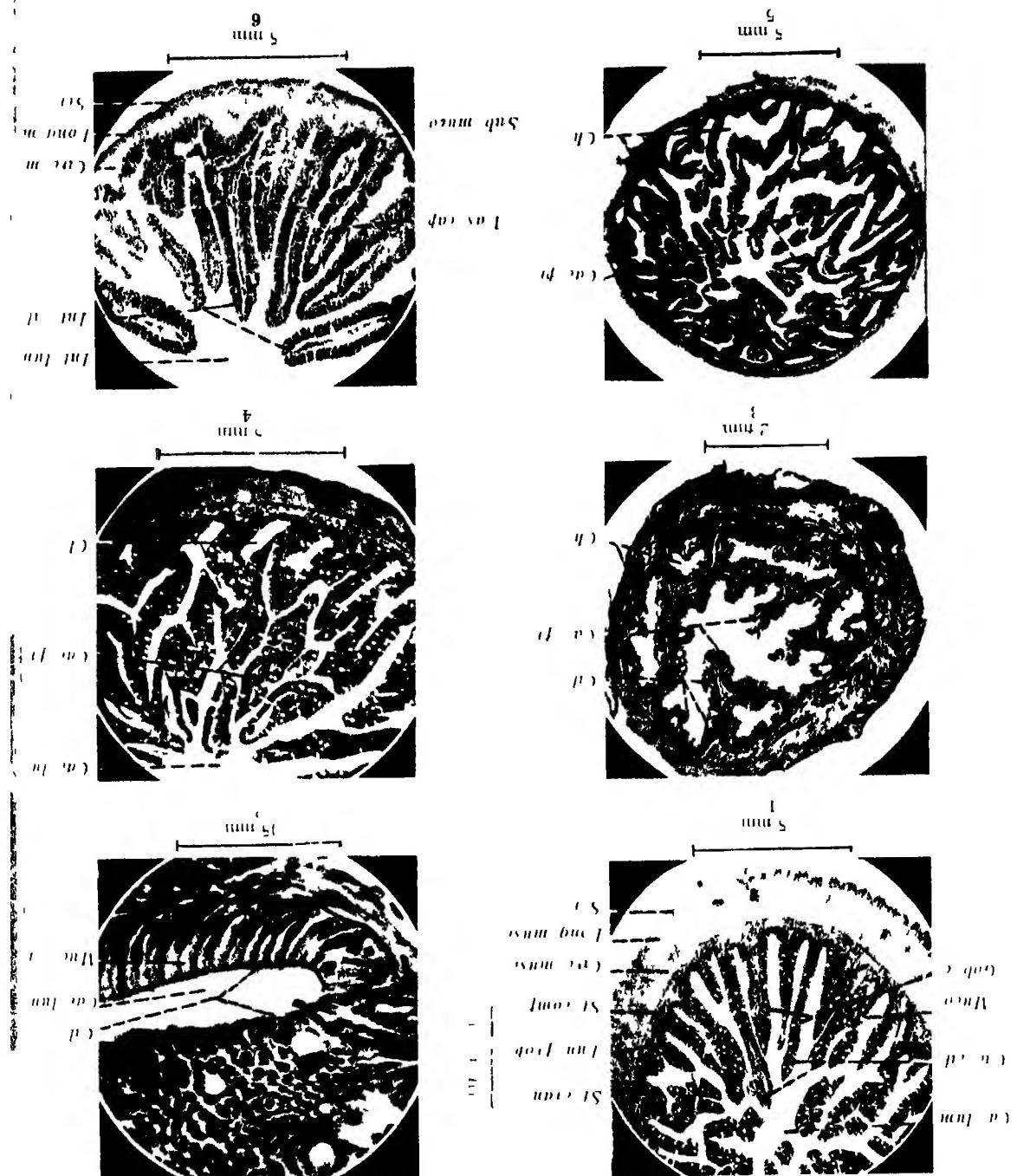
Photo-

- micrograph 7 TS of the proximal part of the cæcum of *Abudedefduf cochinensis* showing pointed, finger-like "cæcal villi"
- . 8 TS of the median portion of the cæcum of *A. cochinensis* showing greater development of the "cæcal villi" and the presence of goblet cells
- . 9 TS of the distal part of the cæcum of *A. cochinensis* showing interdigititation of the "cæcal villi"
- . 10 TS through the tip of the cæcum of *A. cochinensis* showing the formation of several inter-communicating channels
- . 11 TS of the proximal part of the cæcum of *Therapon jarbua* showing the arrangement of the "cæcal villi."
- . 12 TS of the distal part of the cæcum of *T. jarbua* showing proliferation and fusion of the "cæcal villi" to form inter-communicating channels

PLATE III

Photo-

- micrograph 13 TS of the proximal part of the cæcum of *Siganus java* showing the disposition of the "cæcal villi"
- . 14 TS of the proximal part of the cæcum of *Hepatus nigricans* showing proliferation of the "cæcal villi" and formation of channels
- . 15 TS of the distal part of the cæcum of *H. nigricans* showing formation of more channels
- . 16 TS of the proximal part of the cæcum of *Holocentrum ruber* showing slightly branched finger-shaped "cæcal villi"
- . 17 TS of the distal part of the cæcum of *H. ruber* showing formation of inter-communicating channels
- . 18 TS of the proximal part of the cæcum of *Pterois russelli* showing the arrangement of the "cæcal villi"



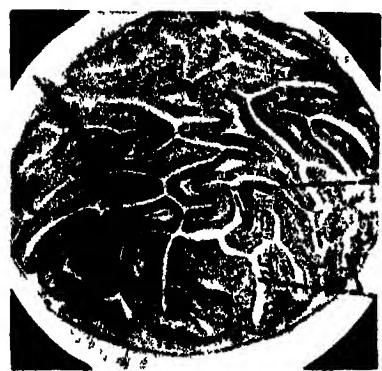
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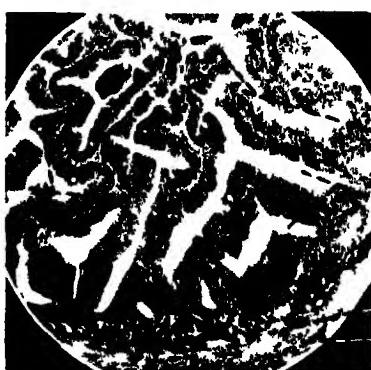
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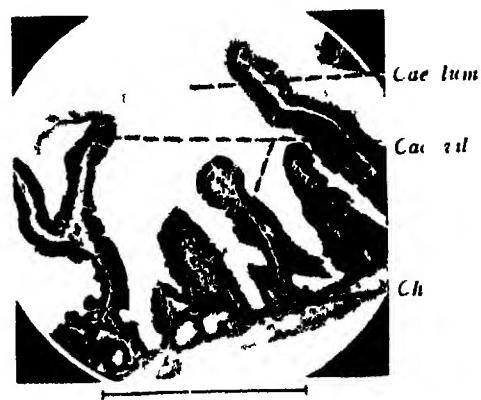
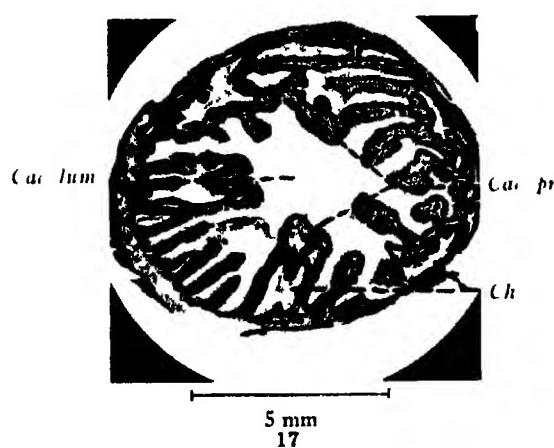
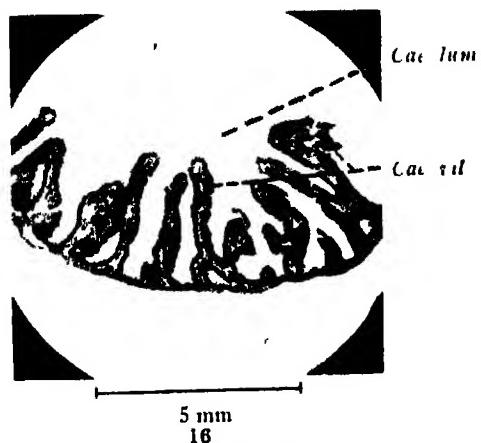
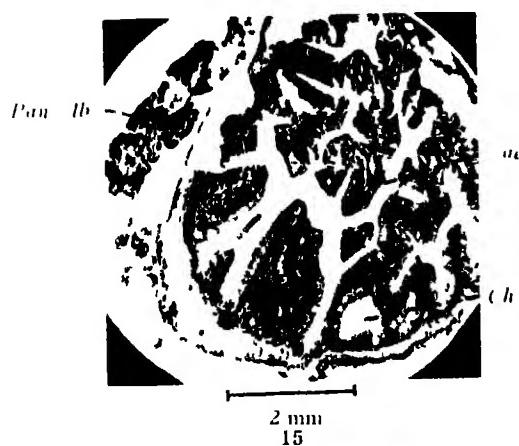
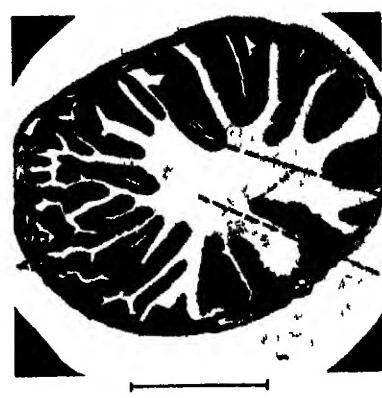
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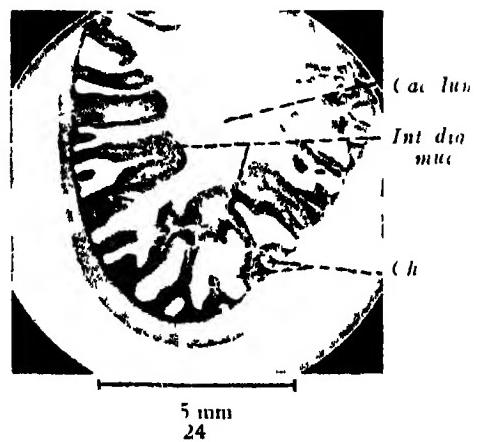
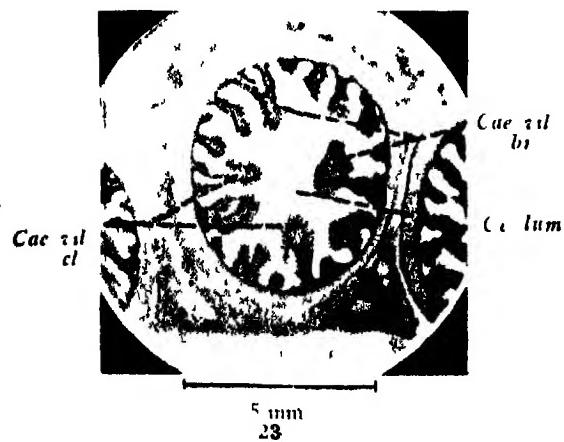
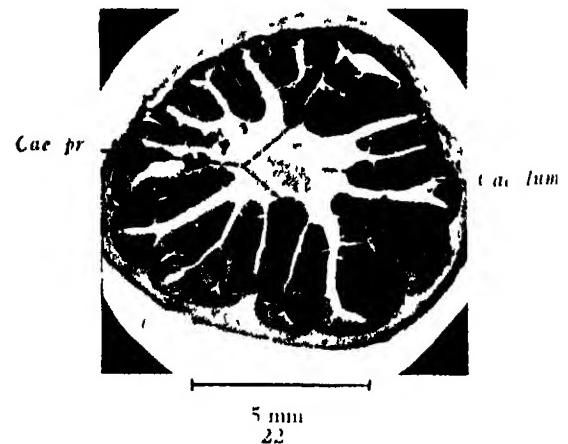
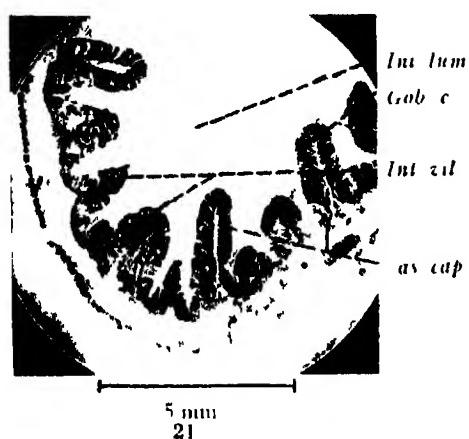
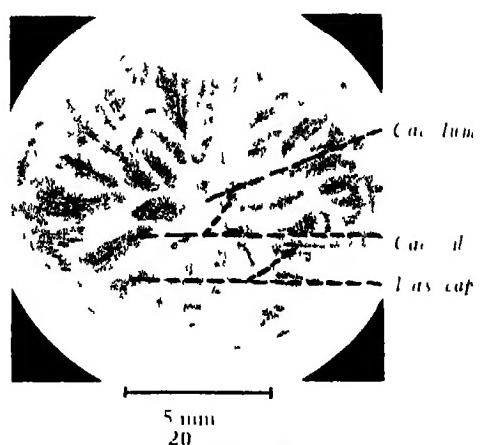
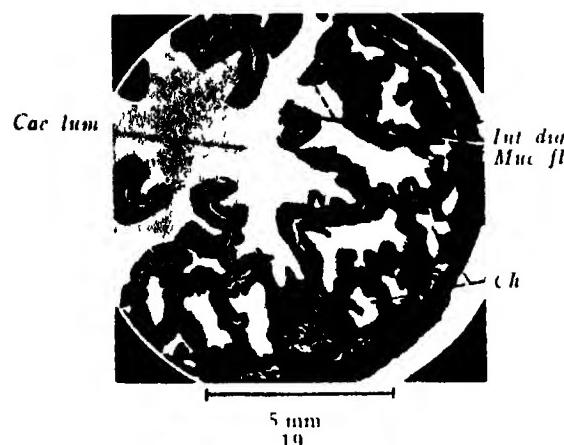


PLATE IV

Photo-

- micrograph 19 TS of the distal part of the cæcum of *P. russelli* showing interdigitations of the "cæcal villi" and formation of the channels
 .. 20 TS of the proximal part of the cæcum of *Leptocheneis naucrates* showing peculiar disposition of the highly vascular "cæcal villi"
 .. 21 TS of the intestine of *Scatophagus argus* showing slightly feathery appearance of mucosal folds and goblet cells
 .. 22. TS of the proximal part of the cæcum of *S. argus* showing conical and club-shaped "cæcal villi"
 .. 23 TS through the proximal region of a single tuft of three small cæcal diverticula of *Serranus tauvina* showing the general disposition of the "cæcal villi"—the median cæcal diverticulum completely cut, and the lateral ones only partially so
 .. 24 TS of the distal region of a single cæcum from the cæcal tuft of *S. tauvina* showing interdigitations of the "cæcal villi" and formation of channels

13. KEY TO LETTERING

Bl dc, Bile-duct , *Cæ*, Cæcum or cæca , *Cæ ar gr*, Group of arboreaceous type of cæca
Cæ bn, Cæcal bunch , *Cæ br*, "Cæcal mop", *Cæ cl*, Cæcal clusters . *Cæ dc*, Cæcal duct ;
Cæ ln, Linear row of cæca , *Cæ ln d*, Anterior linear row of cæca , *Cæ ln v.*, Posterior linear row of cæca , *Cæ lum*, Cæcal lumen , *Cæ m*, Compact gland-like cæcal mass , *Cæ muc fl*
 Folds of the cæcal mucosa , *Cæ op*, Cæcal openings , *Cæ pr*, Proliferating "villi" of the cæcum , *Cæ ros*, "Cæcal rosette", *Cæ spl*, "Cæcal spiral", *Cæ vil*, "Cæcal villi", *Cæ vil br*, Branched "cæcal villi", *Cæ vil cl*, Club-shaped "cæcal villi". *Cæ wl*, "Cæcal whori". *Ch*, Inter-communicating channels inside the cæcal lumen . *Cil*, Cilia , *Circ muc*, Layer of circular muscle-fibres ; *F tis*, Fatty-tissue , *Gl bl*, Gall-bladder , *Gob c*, Goblet cells , *Gs bl*, Gas-bladder , *Int*, Intestine , *Int dig muc fl*, Interdigitating mucous folds , *Int lum*, Intestinal lumen , *Int vil*, "Intestinal villi", *L cæ gr*, Left cæcal group , *Liv*, Liver , *Long muc*, Layer of longitudinal muscle-fibres , *Muco*, Mucosa , *Muco ep*, Cells of mucous epithelium , *Musc*, Muscles , *Oes*, Oesophagus , *Pano lb*, Pancreatic lobules cut , *Pyl*, Pylorus , *R cæ gr*, Right cæcal group ; *Ser*, Serosa ; *St*, Stomach , *St comp.*, Stratum compactum , *St gran*, Stratum granulosum , *Sub muc*, Sub-mucosa , *Tun prop.*, Tunica propria , *Vas cap*, Vascular capillaries

GALL FORMATION IN PADDY

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Received August 24, 1944
(Communicated by Prof D. D. Karve)

GALL FORMATIONS of the nature of long hollow tubes are met with in paddy when the crop is attacked by a midge *Pachydiplosis-oryzae* Mani. The colour of the 'tubes' is either silvery green or similar to the colour of the leaf-sheath of the particular variety of the plant. In attacked plants the normal panicle is destroyed and the farmer suffers a great loss.

This kind of damage in paddy is met with in Indo-China, Malaya, Philippines, Sumatra, Java, Ceylon, Burma and India. In India it is found in Madras, Central Provinces, Orissa, Bihar and Bengal. In the Central Provinces, where this work was completed, these galls were seen on the paddy crop from the middle of June till the end of August, i.e., from the seedling stage up to the time the crop is about to ripen. The affected plant on getting some extra nutriment, gives out adventitious shoots, which may bear a panicle. If the attack is severe, then these secondary shoots are also affected.

Some work has been done on the Zoocecidia and the Cecidia in plants of different Natural Orders, but little attention has been paid to the galls on Graminæ, and especially this particular gall on *Oryza sativa* Linn.

Rao (1919) was the first entomologist to devote his attention to grass galls in India. Rejuvan and Von Leewen (1912) have studied a number of galls in Graminæ, and have later (1926) mentioned that the deformed galls of *Cynodon dactylon* Linn were probably the malformed leaf-sheaths. In spite of these works, no explanation is yet available as to the exact nature of the formation of galls by insects in Graminæ, and particularly in paddy. This paper deals with a gall on paddy formed by *Pachydiplosis oryzae* Mani, and puts forward a simple explanation of the nature of its formation.

This work was done during the pendency of a scheme financed by the Imperial Council of Agricultural Research for the investigation of the 'Gangai' disease of paddy in the Central Provinces. The author expresses his deep sense of gratitude to the authorities of the Department of Agriculture, C.P., for the facilities given, and to Mr. M. S. Mani and Prof. S. L. Ajrekar for their valuable suggestions. I am much thankful to Dr. D. D.

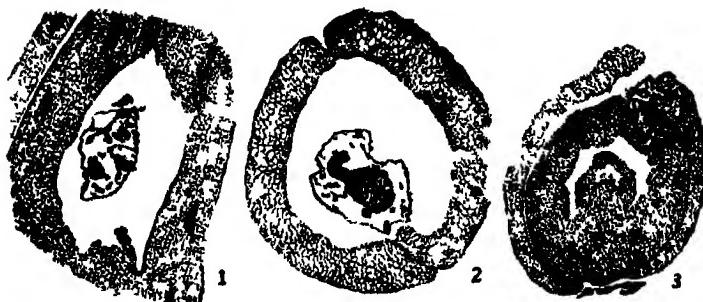


FIG 1 Longitudinal section of a young gall with the maggot in the centre

FIG 2 Transverse section of the gall taken at a lower level, showing the maggot in the centre

FIG 3 Sagittal section of a normal paddy plant near the growing point of the shoots

Karve for seeing this paper through the press. The drawings were kindly drawn by Mr H V Kashyap. A very brief note on this aspect was already published (Deoras, 1941), and the present paper is aimed at stimulating work in this interesting subject.

THE 'SILVERY TUBE'

This is the gall which is a hollow structure. It is completely round at the base and tapering at the tips. A green leaf-blade is generally seen attached to the tips. Between the base of the blade and the tip of the tube, are awl-shaped ligules, and the sickle-shaped auricles. The maximum length of the gall was seen to be about 18 inches, and the girth to be $\frac{1}{4}$ inch. In well-developed galls a small hole was often seen at the tip, and near this lay the moulted skin of the pupa of the midge. In the galls which did not show this exit hole, a pinkish pupa was seen inside. In very tiny galls, which are not apparent unless searched, a very small maggot, pink in colour was observed at the base of the gall. Such tiny galls were hardly 2 mm in length. By the time the gall is visible from between the leaf-sheaths, the pupa had either escaped or was near the tip of the gall.

HOW THE 'SILVERY TUBE' (GALL) IS FORMED

It has been observed that the insect lays eggs under the surface of the paddy leaves. The maggots that hatch, crawl down the grooves of the leaf-surface, and reach up to the very bases of the leaf-sheaths. The passage of the maggot from here to the inside of the stem is rather difficult to follow, except in serial sections. Once inside, the maggot feeds upon the soft tissues there.

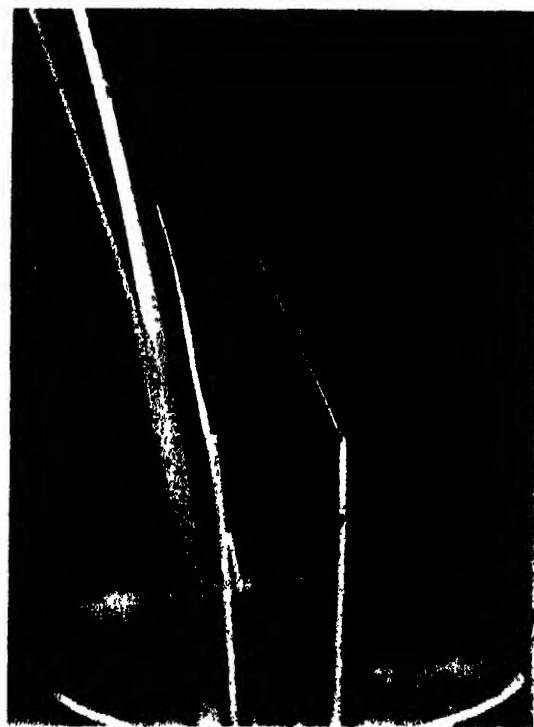
During the growth of the normal paddy shoot, the innermost part of the growing point (*i.e.*, of the terminal bud), which will become the panicle, is the last to grow. Round about this and above this, are the different leaves which grow one after the other, and remain appressed to one another. Each leaf as it grows, distends the leaf-sheath of the previous leaf, the auricles are separated, and the ligule set aside. It is the physiological stimulus of the force exerted by the growing shoot that opens the enclosing ring formed by the preceding leaf-sheath, its ligule, and the auricle. So long as this stimulus is not felt by the leaf-sheath, it will not open the enclosure.

In an attacked plant, the larva has invaded the softest part of the shoot, *i.e.*, the growing point. The leaf-sheaths behind the growing point (now affected) may be the panicle or some shoots preceding it. Nutriment is there, and is being directed to all the parts. The sheaths referred remain normally in a rolled up state. Normally the affected shoots would have opened this rolled up sheath, by growing through them. But as they are affected, they do not grow. The maggot is in their growing region and is constantly feeding all over this area, thus interfering with the growth of the shoot in a purely mechanical manner. The growth as is apparent under these circumstances is that of the rolled up leaf-sheath preceding the affected growing point. The plant is also reacting to the wounds and a vigorous growth is maintained in this rolled up leaf-sheath. The overlapping ends of the leaf-sheath grow into each other. The fusion of these ends may be brought about by the tissues getting meristematic in the region. The activity of the cells thus increased may be due to the pressure which is being exerted on them by the active growth of the sheath, whose ends are in contact for a longer time than normal, a well-known phenomenon in Teratology. The auricles also help in keeping the sheath closed. Thus a closed leaf-sheath grows like an empty tube carrying with it the blade, the auricles and the ligule. The growth of the new shoot is hampered by the insect larva. No more shoots grow after the 'tube', as such the physiological stimulus of force exerted by the growing shoot is absent. This force opens the rolled up leaf-sheath, and in its absence the sheath remains closed. The ends of the closed leaf-sheath grow into each other by merely the pressure of growth as stated above. Longitudinal and transverse sections taken serially illustrate this view.

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Photograph of actual galls as they appear in nature (each gall is mirrored).

SPERMATOGENESIS OF *LACCOTREPES GRISEUS*, GUER.

BY MISS C K RATHNAVATHY, M Sc.
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Received April 18, 1944
(Communicated by Prof R Gopala Aiyar)

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INTRODUCTION

CONSIDERABLE attention has from time to time been directed to the study of spermatogenesis in various species of Hemiptera. Earlier workers were content to follow in general the changes during sperm formation while the more recent workers have concentrated their attention mostly on the behaviour of the chromosomes, the golgi, and the mitochondria. Literature on the hemipteran spermatogenesis is so vast that no detailed account of previous work can be attempted here though mention may be made of the more important contributions towards the subject.

Amongst the most notable accounts are those of Wilcox (1895) on *Cicada*, Montgomery (1897 and 1898, and 1910 and 1911) on *Pentatoma* and *Euschistus* respectively, Paulmier (1899) on *Anasa tristis*, Gross (1904 and 1906) on *Syromastes* and *Pyrrhocoris*, Wilson (1905, 1906, 1907, 1908, 1909, 1910, 1911, 1912, 1913) on the chromosomes of forms like *Lygaeus*, *Coenus*, *Euschistus*, *Nezara*, *Metapodus*, *Banasa* and *Pentatoma*, Tannreuther (1907) on the "History of the germ cells and early embryology of certain Aphide", Foot and Strobell (1907) on "The accessory chromosome of *Anasa tristis*" and Wilke (1907) on the spermatogenesis of *Hydrometra*. Not less noteworthy are the accounts of Browne (1913) on *Notonecta*, Divaz (1915) on the spermatogenesis of *Naucoris cimicoides* and of Honda (1920) and Shaffer (1920) on the spermatogenesis of Aphids and *Cicada septemedecim* respectively. The most remarkable contributions however are the series of studies on insect spermatogenesis by Bowen (1920, 1922 a, 1922 b, 1922 c, 1922 d and 1922 e) dealing mostly with the problem of the behaviour of the golgi and the mitochondria during spermatogenesis as noted in *Euschistus*, *Brochymena* and *Murgantia* as also with other problems such as "the phenomena of polymegaly in the sperm cells of the family Pentatomidae" and "The occurrence of abnormal mitoses in spermatogenesis," and the like. Other accounts requiring mention are those of Spaul (1922) on "The gametogenesis of *Nepa cinerea*," Payne, F (1927) on "Some cytoplasmic structures in the male germ cells of *Gelastocoris oculatus*" and Payne, M A (1934) on "Intravitam studies in *Leptocoris trivittatus*" as also, that of Poisson (1927) and Voinov (1927) on the male sexual cells of *Notonecta glauca*. Other noteworthy contributions are those of Chickering (1927, 1932) on the "Spermatogenesis in the Belostomatidae" and of Chickering, Howard and Howes, on "The spermatogenesis of *Ranatra fusca*," Steopoe (1927 and 1931) on the spermatogenesis of *Ranatra linearis* and *Nepa cinerea* Pollister (1930) on the "Cytoplasmic phenomena in the spermatogenesis of *Gerris*," Schrader (1931) on "The chromosome cycle of *Protortonia primitiva*" and Gatenby (1931 c) on "The post-nuclear granule in *Anasa tristis*".

The present paper deals with the spermatogenesis of the common Indian water bug, *Laccotrephes griseus* and gives a complete account of the behaviour of the chromosomes, golgi apparatus, mitochondria and centrosome. The work was done during the period extending from July 1937 to August 1938 the forms being examined at very close intervals. Stages of the bug at the time when they are wingless up to the stage of the adult have been carefully examined, the younger forms killed in their last instar showing all the stages of spermiogenesis very distinctly.

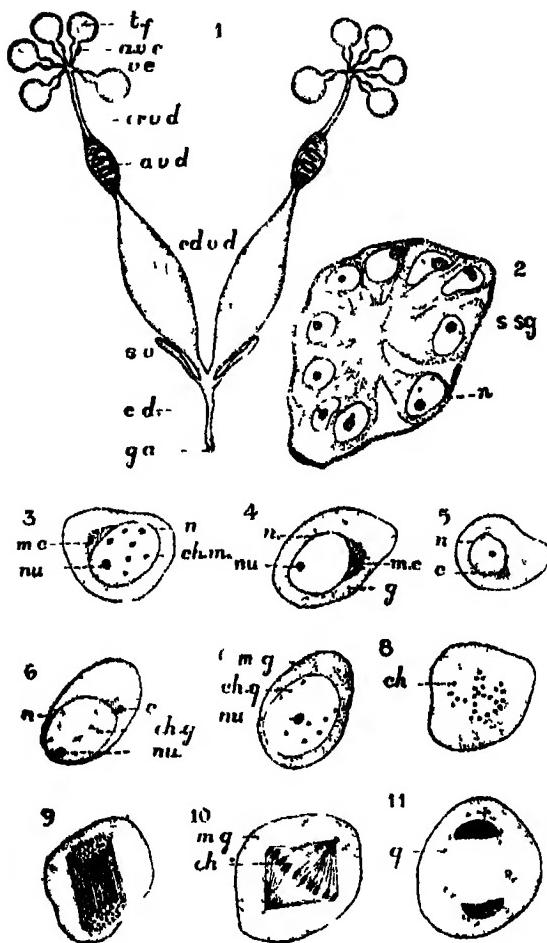
Laccotrephes griseus, commonly known as the water scorpion, belongs to the sub-order Heteroptera of the order Hemiptera. It is obtained in large numbers from tanks and pools in and about Madras. The only other species of *Laccotrephes* that occurs in Madras is *ruber* which is distinguished from *griseus* by its abdomen being coloured distinctly red.

MATERIAL AND METHODS

The material consisted in the testis of the water scorpion *Laccotrephes griseus*, young forms which are wingless, as well as adult winged forms being examined in large numbers both in the living and fixed conditions. The forms were dissected in normal saline solution and the reproductive organs rapidly transferred to the various fixatives after being completely cleared of their surrounding tissue. The fixatives employed consisted in the following fluids --

Corrosive sublimate acetic,
Carnoy's fluid,
Flemming with acetic,
Bouin's fluid,
Schaudinn's fluid,
Champy's fluid,
Guthrie's strong flemming,
Flemming without acetic,
Zenker's without acetic,
Benda's fluid,
Mann-Kopsch fluid and its modification by Ludford,
Nassanov's fluid,
Kolatchev's fluid,
Dafano's fluid

Chromosomal work chiefly consisted in fixation in Bouin's fluid and Flemming with acetic and staining with Ehrlich's haematoxylin and eosin, and iron haematoxylin and Orange G. These preparations were useful for studying the details of spindle fibres, centrosome and nucleolus as also for obtaining precise pictures of the chromosomes. Carnoy's fluid was found to be useful for the study of chromosomes and centrosomes while corrosive sublimate acetic and Schaudinn's fluid rough and unreliable in their fixation. For studying the true nature of the nucleolus Feulgen's "Nucleal farbung" method was employed as also fixation by Zenker's without acetic and corrosive acetic followed by Mann's methyl-blue eosin stain, besides fixation by Bouin's and Flemming's followed by iron haematoxylin. For the study of the centrosomes Denne's modification of iron-alum haematoxylin was employed and this was found to give very successful results after fixation in Bouin's for about half an hour and in Carnoy's for ten minutes. Chrome osmium fixatives such as Flemming without acetic and Champy were used frequently and Gatenby's modification of Flemming strong fluid without acetic was found to demonstrate the cytoplasmic elements such as



All figures except 1, 52, 53 and 54 are drawn with a camera lucida at table level using a Zeiss microscope

Figs 1-11—Fig 1 The male reproductive system Diagrammatic Fig 2 Cross-section of a spermatogonial cyst showing the arrangement of the secondary spermatogonia in the form of rosette, Carnoy and iron haematoxylin Fig 3 Resting primary spermatogonium showing the mitochondrial cap Flemming without acetic and iron haematoxylin $\times 2000$ Fig 4 Resting primary spermatogonium showing the mitochondrial cap and Golgi bodies Mann-Kopsch and Altmann stain $\times 2000$ Fig 5 Resting primary spermatogonium showing the centrosome Carnoy and iron haematoxylin $\times 2000$ Fig 6 Spermatogonial prophase Carnoy and iron haematoxylin $\times 2000$ Fig 7 Later spermatogonial prophase Mitochondrial granules scattered in cytoplasm Champy and iron haematoxylin $\times 2000$ Fig 8 Polar view of spermatogonial metaphase Carnoy and iron haematoxylin $\times 2000$ Fig 9 Spermatogonial anaphase Carnoy and iron haematoxylin $\times 2000$ Fig 10 Spermatogonial metaphase Distribution of mitochondrial granules Flemming without acetic $\times 2000$ Fig 11. Spermatogonial anaphase Distribution of Golgi bodies Mann-Kopsch $\times 2000$

the Golgi and mitochondria distinctly. In connection with these chrome osmium fixatives Heidenhain's iron haematoxylin was used, the mitochondria staining very intensely in both cases. After Champy fixation, Champy Kull's acid fuchsin, thionin and aurantia stain was used, the mitochondria in this case staining pink. Benda's alizarin method was also employed after hardening besides staining with iron haematoxylin. Guthrie's strong Flemming was also employed followed by staining in crystal violet according to Benda's alizarin method. This gave splendid results picturing the mitochondria distinctly in the spermatogonial, spermatocyte and spermatid stages. Of the formaline-chrome-techniques Regaud's formol bichromate and iron haematoxylin was attempted with fair success.

To study the Golgi apparatus resort was taken to the method of Mann-Kopsch as also its modification by Ludford, the Kolatchev method as modified by Nassanov, and also method of Cajal. The last method did not yield very satisfactory results while the others gave most splendid results, the method of Mann-Kopsch and Ludford excelling that of Nassanov.

Paraffin method of embedding was employed in all cases and sections were cut ranging in thickness from three to ten microns. Of these the thinner sections were found to be more useful in studying the cytoplasmic elements while the thicker sections provided satisfactory pictures of the nuclear constituents.

Smears were prepared fixed in Bouin, Carnoy, Flemming with and without acetic and Champy and these proved very useful for work on both nuclear and cytoplasmic elements. The structure of the sperm was carefully studied by means of these smears.

Vital staining by Janus green and Neutral red was also attempted and the mitochondria in the spermatids clearly studied by this method. The Golgi bodies distinguished themselves with great difficulty and so also the mitochondria of the earlier stages of spermatogenesis.

THE MALE REPRODUCTIVE SYSTEM

The male reproductive system of *Laccotrephes griseus* (Fig 1) consists of a pair of testes situated in the second segment of the abdomen, each made up of five follicles, of which four remain grouped together and one lies separate, the latter being slightly larger and inclined towards the median line. The follicles of each testis are round or slightly oval in shape and each at its base is continued into a thin, short vas efferens which exhibits a globular structure along its course known as the ampulla of the vas efferens. From the lower end of each ampulla, the vas efferens proceeds a short way when all

the five vasa efferentia join together to form the vas deferens on each side. The vas deferens at about a third of its length has a small dilated structure which is formed by the duct taking a spiral course. This is known as the ampulla of the vas deferens. The region of the vas deferens anterior to the ampulla is known as the cranial region of the vas deferens and the region posterior to the ampulla the caudal region of the vas deferens, the latter being much dilated in mature forms. The vasa efferentia join together posteriorly to form a narrow ejaculatory duct which opens to the exterior by the male genital aperture. Just at the proximal end of the ejaculatory duct a small tubular seminal vesicle is given off on each side. The seminal vesicles lie closely adherent to the sides of the dilated regions of the vasa efferentia and are never seen to be distended with sperms, the posterior halves of the vasa efferentia acting as storage places for the mature sperms before they are finally passed to the exterior through the male genital aperture. The genital opening is guarded by two pairs of chitinous plates enclosing four styliform processes all of which together form the intromittent organ of the animal.

THE TESTICULAR FOLLICLES

Each follicle of the testis is composed of long convoluted tubules containing the seminal elements in various stages of development. Enveloping the follicles of the testis and continuous with the vas deferens is a layer of connective tissue in which are somatic nuclei, trachea and certain red pigment granules which make the testes appear red at a certain period of their development. Just internal to this layer extends a band of fibrous tissue which is very prominent in cross-sections of young testicular follicles. The innermost lining of the follicle consists of a thin layer of cells containing deeply staining nuclei. When the cross-section of a well advanced follicle is examined its interior is seen to be divided into compartments each compartment representing the cut end of a testicular tubule. Within each tubule may be distinguished groups of cells enclosed by surrounding walls. These are called cysts. The cells contained in a cyst are all found to be almost at the same stage of development and there is always present an almost serial gradation of stages from one end of the tubule to the other, that is, starting from the anterior end of each tubule there are found to be the spermatogonia, then the spermatocytes, later the spermatids and lastly the fully mature sperms.

THE SPERMATOGONIAL STAGES

The blunt ends of the follicular tubules in the testis of *Laccotrephes griseus* are filled with isolated spermatogonia. These isolated cells further

down occur in groups, each group being separated from the rest by surrounding walls of tissue. The isolated spermatogonia may be called the primary spermatogonia while those that occur in the cysts, the secondary spermatogonia, each group of the latter being formed as a result of the division of a single primary spermatogonium. Each young cyst (Fig 2) in a cross-section, is seen to consist of eight to ten cells which are conical in shape and grouped in the form of radial clusters or rosettes. The number of cells in each cyst varies slightly though in a given spermatocyst the cells are all approximately in the same stage of development. The cells are arranged with their apices pointing towards the centre of the cyst while their bases lie fringing the periphery of the cyst. The inner ends of these spermatogonial cells are more or less united one with the other. This cytoplasmic continuity of cells is suggestive of young secondary spermatogonial cysts. In a slightly older cyst this median connection between the spermatogonia is interfered with and each cell now becomes less conical after separation from its sister cells.

The resting spermatogonia are thus irregular conical cells (Figs 3 to 5) the cytoplasm of which presents mitochondria and Golgi bodies besides a definite centrosome. The mitochondria in the resting spermatogonium occur as a crescentic cap closely applied to the nuclear wall (Figs 3, 4). This mitochondrial cap is clearly demonstrated in material fixed in Champy, Flemming without acetic and Benda's fluid and stained by iron haematoxylin. This mitochondrial cap when closely examined, has a finely granular appearance. The Golgi bodies (Fig 4) appear as small crescentic structures in the region of the mitochondrial cap and each is seen to be composed of an outer chromophilic part partly enclosing a chromophobic area. No definite compact idiosome is present in this form, the idiosome being represented by many separate masses of idiosomic material each accompanied by a curved Golgi rodlet, both together constituting a so-called Golgi body. The centrosome (Fig 5) appears as a distinct darkly-staining granule lying in the immediate vicinity of the nuclear wall.

The nucleus of the resting spermatogonium (Fig 3) is fairly large in comparison with the surrounding cytoplasm and is situated at the large end of the cell. Chromatin occurs in the form of a few scattered, irregular masses within the nuclear cavity. The spermatogonia are about the smallest in size considering the various cell generations that occur in the testis, and each spermatogonium is surrounded by a very delicate cell wall.

With the commencement of the prophase of mitosis, the chromatin masses break up into a number of small granules (Fig 6) which enlarge

until finally each attains an irregular spherical shape (Fig 7). Within the cytoplasm, the mitochondrial cap gradually gets disrupted with the result that a cloud of mitochondrial granules is produced which spreads throughout the cytoplasm (Fig 7) The Golgi bodies also get dispersed throughout the cytoplasmic area During this period, the centrosome divides and the divided products of the centrosome travel apart from each other until they occupy the two distal ends of the cell A spindle is soon formed between the two centrosomes, the nuclear membrane making its disappearance meanwhile The irregularly spherical chromatic masses, described in the spermatogonial prophase, now attain the size of the ordinary chromosomes of the spermatogonia and become more spherical in shape These spherical chromosomes are caught up in the spindle fibres and the metaphase figure is formed The spindle of the spermatogonial metaphase is short and the astral fibres around the centrioles are not distinct The centrioles show their best staining capacity at the time the division figures are formed occurring as distinct granules at the polar ends of the spindle A cross-section of the equatorial plate (Fig 8) shows that there are forty-two chromosomes though the exact identification of the idiochromosomes merely from their size is impossible at this stage However, the capacity of the sex chromosomes to stain deeply even in the early spermatogonial stage helps to reveal their identity on the spindle

During the metakinesis each one of the chromosomes divides simultaneously into two parts which separate to the two spindle poles, the spindle figure at the late anaphase stage taking on the form of a short stout cylinder (Fig 9) With the division of the spermatogonia the mitochondria get approximately divided between the two daughter cells (Fig. 10) The Golgi bodies too get distributed in a like manner there being no special mechanism for the equal division of the Golgi bodies to the two daughter cells (Fig 11). However, the orientation of the chondriosomes and Golgi bodies in larger numbers around the centrosomes during division seems to indicate that the centrosomes exert some influence on the distribution of these cytoplasmic structures

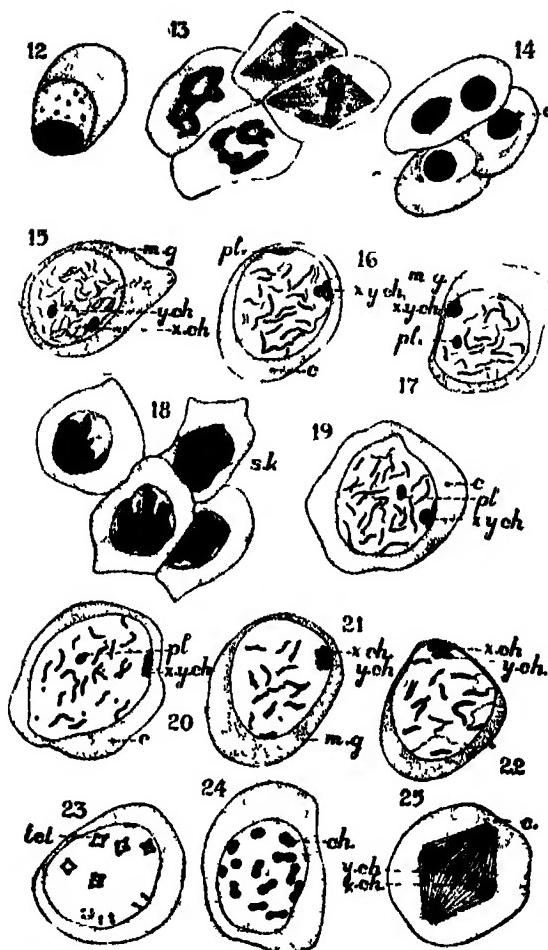
THE NUTRITIVE CELLS

In the region of the spermatogonia all the cells of certain cysts undergo a process of degeneration, these probably serving as nutritive material for the rest of the developing cells. The cells in this region are seen to undergo degeneration at two or three stages in their developmental history In the first case the chromatin present in the nucleus of certain spermatogonial cells that are on their way to development into spermatocytes gather in the

form of lens-shaped masses on the inner wall of the nucleus (Fig 12) until finally the whole nucleus is filled with such chromatin masses, and stains intensely dark with haematoxylin. The cells thus arrested in their further growth, get clumped together in a cyst, the whole cyst degenerating ultimately. In the second case, certain spermatogonia pass through their prophase stages, but when the chromosomes reach the metaphase condition they fail to divide and form one chromosome band which lies more or less twisted on the metaphase spindle (Fig 13). No division of the chromosomes takes place and the entire chromatin band gets rounded the spindle fibres meanwhile degenerating gradually. The centrosomes are never distinct in such cells. In the third case, degeneration commences at a slightly later stage. The metaphase spindle is here developed and division of the chromosomes takes place, but after division no reconstitution into the spermatocyte nucleus takes place degeneration commencing soon after the chromosomes on the spindle have divided (Fig 14). In such cases the degenerated cells are very small and the centrosomes are distinct. Such degenerating cells are not met with in any other part of the testis except where the spermatogonia and spermatocytes exist and the fact that such a phenomenon occurs in the region of greatest growth seems to suggest that these degenerating cells serve as nutritive material to the normally developing cells of the adjoining cysts. Such nutritive cells have been met with in testes of nymphs as well as of adults and they are present in almost every individual.

THE SPERMATOCYTIC STAGES

As a result of the final secondary spermatogonial division the primary spermatocyte is produced which does not undergo a period of rest. This cell, unlike the spermatogonium, has more or less a polygonal shape and the spermatocytes in a particular cyst are not arranged in the form of rosettes. Its cytoplasm is very much reduced compared to the amount of cytoplasm present in the spermatogonium, the nucleus being very much larger in size and occupying almost the whole of the interior of the cell. Within the nucleus a gradual lengthening of the chromatin elements now takes place resulting in the formation of the leptotene threads (Fig 15). However, the idiochromosomes or the sex chromosomes do not undergo this process of elongation but remain as compact bodies and lie within the nuclear space independent of each other (Fig 15). These sex chromosomes retain this position only in the very early stages of the growth period. Of the sex chromosomes one is distinctly larger than the other and as the leptotene stage advances the larger sex-chromosome comes to lie adjacent to the nuclear wall while the other takes an inconstant position within the



Figs 12-25—Figs. 12-14 Nutritive cells Carnoy and iron haematoxylin $\times 2000$
 Fig 15 Primary spermatocyte Leptotene nucleus X and Y chromosomes lie independent of each other Mitochondrial granules scattered in cytoplasm Flemming without acetic and iron haematoxylin $\times 2000$ Fig 16 Primary spermatocyte Zygote nucleus Apposition of X and Y chromosomes Appearance of plasmosome Carnoy and iron haematoxylin $\times 2000$ Fig 17 Primary spermatocyte Zygote nucleus Plasmosome migrating towards the interior of nucleus Mitochondrial granules scattered in cytoplasm Flemming without acetic and iron haematoxylin $\times 2000$ Fig 18 Primary spermatocytes Synaptonemal nuclei Carnoy and iron haematoxylin $\times 1000$ Fig 19 Primary spermatocyte Zygote nucleus Elongation of the X chromosome. Carnoy and iron haematoxylin $\times 2000$ Fig 20 Primary spermatocyte Pachytene nucleus Elongation of Y chromosome and conjugation between the X and Y chromosomes Carnoy and haematoxylin $\times 2000$ Fig 21 Primary spermatocyte Diplotene nucleus Separation of X and Y chromosomes and their transformation into dumbbell-shaped structures Mitochondrial granules scattered in cytoplasm. Flemming without acetic and iron hematoxylin $\times 2000$ Fig. 22 Primary spermatocyte Diplotene nucleus Further separation of X and Y chromosomes and their transformation into dumbbell-shaped structures. Carnoy and iron

haematoxylin $\times 2000$ Fig 23 Primary spermatocyte Tetrad well developed Carnoy and iron haematoxylin $\times 2000$ Fig 24 Primary spermatocyte Formation of dumbbell-shaped chromosomes Carnoy and iron haematoxylin $\times 2000$ Fig 25 Primary spermatocyte Side view of metaphase Carnoy and iron haematoxylin $\times 2000$

nucleus, sometimes lying against the nuclear wall. Besides this characteristic size difference in the sex chromosomes and the retention of their compact forms they possess the character of taking on an intensive stain as was observed from their very early occurrence in the spermatogonia. This staining capacity they retain throughout the stages of both the primary and secondary spermatocytes.

At the period of formation of the leptotene threads and the distinction of the idiochromosomes in the form of two compact deeply staining bodies, a plasmosome makes its appearance for the first time as a flattened disc on the inner surface of the nuclear membrane. It comes into prominence as the nucleus reaches the zygotene stage (Fig 16). The position of the plasmosome is seen to vary on the nuclear membrane except for the fact that it is never seen in association with the idiochromosomes. When the nucleus passes through the zygotene stage the plasmosome loses its connection with the nuclear membrane and shifts its position to the interior of the nucleus (Fig 17), but at a slightly later stage when the nucleus steps into the diplotene condition the plasmosome makes its disappearance.

The leptotene threads, before they pass into the zygotene condition become grouped together in the centre of the nucleus. Thus the synizesis stage which is observed to be very pronounced in this form is reached. The leptotene threads, during the synizesis stage, shorten and thicken to such a remarkable extent that this stage is represented by big black blotches of chromatin within the nuclei. The synizetic knot however soon loses its central position and occupies an excentric area within the cell (Fig 18). During the period of synizesis the chromosomes exhibit such an intense avidity for the stain that the recognition of the sex chromosomes is impossible at this stage. After this period the chromosomes emerge from the synizetic knot and the autosomes lay themselves in parallel pairs within the nucleus (Fig 19). The zygotene stage is thus seen to follow closely the leptotene with the intervention of a period of synizesis. The cell has meanwhile entered on its period of growth. During the pachytene stage (Fig. 20) the parallel chromosomes conjugate along their length and shortly after that there is a slight contraction of the conjugated pairs or gemini, this contraction being apparently due to a twisting of the univalents about each other rather than to any linear contraction. Within the zygotene nucleus the idiochromosomes, that remained independent in the leptotene stage, come to lie closely

apposed to each other against the nuclear membrane (Figs 16, 17 and 19), the apposition between the two sex-chromosomes being effected by an advance of the smaller idiochromosome towards the larger one, the latter being persistently found to lie against the nuclear membrane even from its very early stages in the primary spermatocyte. Once the apposition between the sex-chromosomes has been effected gradual elongation of the larger sex-chromosome takes place, the smaller retaining its rounded appearance (Fig 19) until a later stage in the synaptic period. When the elongation of the larger sex-chromosome has reached its maximum limit the smaller one begins to elongate though this does not attain the length of the larger sex-chromosome (Fig 20). This behaviour of the sex-chromosomes characterises the early pachytene nucleus. Closely following the pachytene stage steps in the diplotene (Fig 21) in which the pachytene threads by a slow process of unravelling appear distinctly double. The diplotene threads next separate at one end, remaining intact at the other, and gradually uncoil themselves free of each other until they appear as distinctly elongated U-shaped structures (Fig 22). A longitudinal splitting now takes place along each arm of the geminus followed by a condensation of the parts, the result being that compact cuboidal bodies are formed within the nucleus. These are the definitive tetrads (Fig 23). The four components of the tetrads now fuse in pairs with the formation of dumbbell-shaped structures which show a definite constriction in the centre (Fig 24), the constriction corresponding to the place of transverse division that is to soon follow. While the autosomes thus undergo the process of tetrad formation and later develop into dumbbell-shaped structures, the idiochromosomes, which in the pachytene stage remained as a conjugated pair of elongated, deeply-staining bodies, separate from each other and each attains a bilobed appearance (Figs 21 and 22). The nuclear membrane at this time ruptures with the formation of the first spermatocyte spindle (Fig 25). The dumbbell-shaped structures are rapidly drawn on to the spindle, these being arranged with the long axes of the dumbbells lying in the plane of the spindle. Of these one is distinctly smaller than the others and may be recognised as the smaller idiochromosome.

The cytoplasmic elements of the primary spermatocyte may now be considered. Within the very early stages of the primary spermatocyte the mitochondria occur in the form of fine granules—the chondriosomes—scattered irregularly in the cytoplasm (Figs 15, 17 and 21). This condition of the mitochondria persists throughout the early prophase stages up to the formation of the diplotene threads in the nucleus. With the initiation of the period of tetrad formation and the formation of the dumbbell-shaped chromosomes, the mitochondrial granules undergo a process of linear fusion

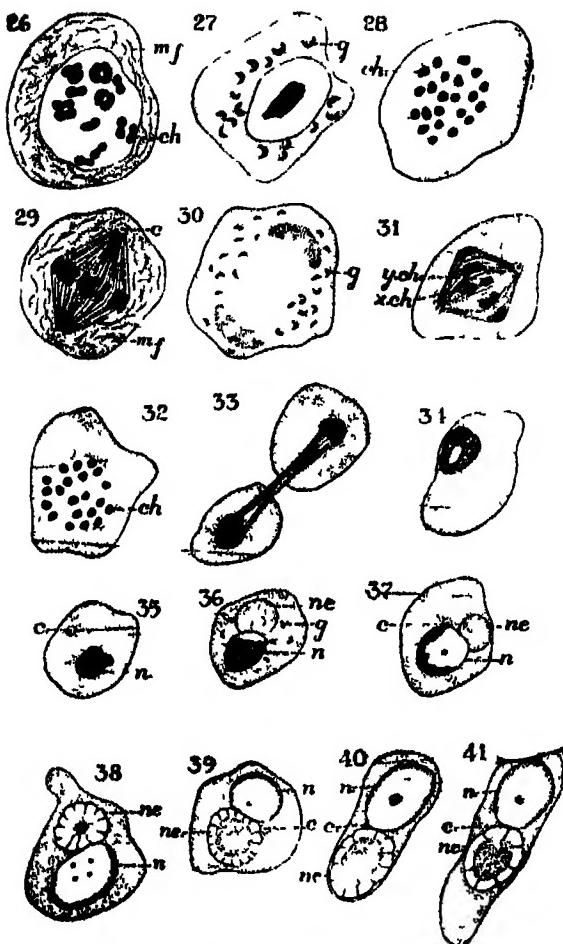
with the result that mitochondrial threads—the chondriocysts—are produced (Fig 26) This filamentous phase of the mitochondria persists throughout all the stages of the later spermatocyte

The Golgi bodies in the primary spermatocyte occur in the form of deep crescents with a chromophilic exterior and a chromophobic interior, scattered uniformly throughout the cytoplasm (Fig 27) In size they are bigger than the Golgi present in the spermatogonia They are easily recognizable in Mann-Kopsch preparations by their characteristic form and contour, besides their intensely blackened appearance due to osmotic impregnation The centrosome can be recognised in the cytoplasm of the spermatocyte as a darkly stained granule (Figs 16, 19 and 20) During the formation of the spindle the centrosome divides and the two products of division migrate to the opposite poles of the cell Around each centrosome a distinct clear area is observed (Fig 25) A polar view of the metaphase plate of the primary spermatocyte always shows twenty-two chromosomes (Fig 28) each of the products of the ensuing division, possessing twenty autosomes and two idiochromosomes the division being reductional for the autosomes and equational for the idiochromosomes The products of division of the primary spermatocyte pass into the secondary spermatocyte spindle without the intervention of a period of rest

The chondriosomes retain their filamentous form during the first maturation division and are sorted out approximately into two equal masses and passed into the two daughter cells (Fig 29) Some of the chondriosomes however appear to be caught midway between the two cells and severed into two No fragmentation of the filamentous forms has been observed The Golgi bodies are also sorted out into approximately equal halves and thus delivered to the two dividing cells (Fig 30)

The secondary spermatocyte does not undergo a period of rest and immediately after the division of the primary spermatocyte the secondary spermatocyte spindle is constituted (Fig 31) In the metaphase spindle of the secondary spermatocyte the idiochromosomes appear in the form of an unequal dyad and during division the twenty autosomes get divided longitudinally while the idiochromosomes pass each to one pole of the spindle (Fig 31) with the result that two kinds of spermatids are produced, one with twenty autosomes and the X-chromosome, the other with twenty autosomes and the Y-chromosome

Therefore in the metaphase of the first division it is certain that there is always one more than the haploid number of chromosomes,—twenty-two (Fig 28) while in that of the second there is the exact



Figs 26-41—Fig 26 Primary spermatocyte with filamentous mitochondria in cytoplasm Champy and iron haematoxylin $\times 2000$ Fig 27 Primary spermatocyte Golgi bodies scattered in cytoplasm Mann-Kopisch $\times 2000$ Fig 28 Primary spermatocyte Polar view of metaphase Carnoy and iron haematoxylin $\times 2000$ Fig 29 Primary spermatocyte Side view of metaphase Distribution of mitochondrial filaments Champy and iron haematoxylin $\times 2000$ Fig 30 Primary spermatocyte Side view of anaphase Distribution of Golgi Mann-Kopisch $\times 2000$ Fig 31 Secondary spermatocyte Side view of very early anaphase Early separation of the idiochromosome dyad Carnoy and iron haematoxylin $\times 2000$ Fig 32 Secondary spermatocyte Polar view of metaphase Carnoy and iron haematoxylin $\times 2000$ Fig 33 Secondary spermatocyte Late telophase Carnoy and iron haematoxylin $\times 2000$ Fig 34 Section across the chromosomal region of one of the division products of the secondary spermatocyte Carnoy and iron haematoxylin $\times 2000$ Fig 35 Early spermatid Carnoy and iron haematoxylin $\times 2000$ Fig 36 Spermatid Formation of nebenkern Golgi bodies present. Mann-Kopisch and Altmann stain $\times 2000$ Fig. 37 Spermatid Flemming without acetic and iron haematoxylin $\times 2000$ Fig 38 Spermatid The nebenkern exhibits a vacuolated appearance. Flemming without acetic and iron haematoxylin $\times 2000$ Fig. 39 Spermatid Differentiation of the

nebenkern into chromophobic and chromophilic zones Flemming without acetic and iron haematoxylin $\times 2000$ Fig 40 Spermatid Migration of centrosome to the region between nucleus and nebenkern Flemming without acetic and iron haematoxylin $\times 2000$ Fig 41 Spermatid Centrosome situated in between nucleus and nebenkern Champy and iron haematoxylin $\times 2000$

haploid number,—twenty-one (Fig 32) In *Laccotrephes* the arrangement of the chromosomes is observed to be irregular on the equatorial plate, the formation of an autosomal ring with the inclusion of only the sex-chromosomes as described in many other Hemiptera, not being observed in this form. However the distinction of the idiochromosomes in the primary spermatocyte is possible in that they always stain deeply besides the fact that they are always placed somewhere within the centre of the chromosomal plate and never at the periphery Within the metaphase of the secondary spermatocyte also a similar condition is observed One point of difference however exists between the anaphases of the primary and secondary spermatocytes whereby the idiochromosomes in the latter cell may be clearly distinguished This consists in the fact that during the division of the chromosomes on the secondary spermatocyte spindle the idiochromosomes precede the division of the autosomes (Fig 31) while in that of the primary spermatocyte they divide alongside with the autosomes (Fig 25) This disjunction of the sex chromosomes before the autosomes is characteristic only of the secondary spermatocyte division The secondary spermatocyte spindle is short and wide and is almost half the size of the spindle of the primary spermatocyte, but when the spindle reaches the telophase stage it assumes a very elongated form (Fig 33) The chromatin at this stage occupies more or less a peripheral position within the nucleus a distinct clear space being visible in the centre (Fig 34) Towards the end of the telophase however, the chromatin clump together in a mass and occur as such in the spermatid nucleus (Fig 35)

Regarding the cytoplasmic elements of the secondary spermatocyte, the centrosome of the preceding division divides precociously so that immediately after the telophase of the first maturation division the secondary spermatocyte constitutes its division figure without the intervention of a period of rest The centrosomes are very distinct at the polar ends of the secondary spermatocyte spindle (Fig 31) and each is surrounded by a colourless area The mitochondria and Golgi occur as scattered elements within the cell and division of these cell components takes place exactly as in the first maturation division

THE SPERMATID

The spermatid is a very small cell compared to the enormous size of the first spermatocyte in its prophase stages It includes a nucleus which has

a dense irregular mass of chromatin in its interior. The Golgi and the mitochondria are present in the cytoplasm the Golgi occurring as a few discrete bodies while the mitochondria exist as a flocculent mass beside the nucleus (Fig. 36). A centrosome is distinct (Figs 35 and 37).

THE TRANSFORMATION OF THE SPERMATID INTO THE SPERMATOZOOON

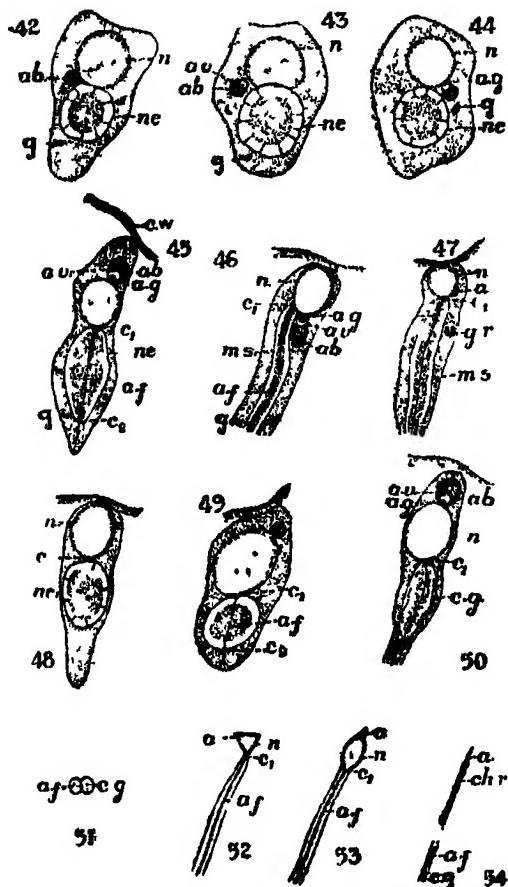
The spermatid soon resolves into its period of development into the mature sperm. The chromatin mass breaks up into irregular fragments which spread throughout the nucleus. Ultimately they get massed into a peripheral band of chromatin which is interrupted at that region of the nucleus which lies in close proximity with the mitochondrial mass (Fig. 37). A few chromatin masses also lie scattered within the nucleus. The nucleus now enlarges greatly in size and the chromatin band gets reduced to a thin homogeneous layer lining the nuclear wall. The mitochondrial mass gets compacted into a spherical body called the *nebenkern* but at this stage it exhibits a very vacuolated appearance (Fig. 38). However it soon gets differentiated into an outer chromophobic region and an inner rounded chromophilic zone. The former presents a vacuolated appearance while the latter exhibits a uniformly homogeneous structure (Fig. 39). As development proceeds the vacuoles of the outer chromophobic zone consistently decrease in number due to the fusion of the vacuoles with each other with the consequent result that the vacuoles steadily enlarge in their dimensions. The final result of this fusion of the vacuoles is the production of one big vacuole which closely envelopes the chromophilic interior. Along with this reduction in the number of vacuoles a gradual enlargement of the chromophilic zone in the centre takes place.

The position of the *nebenkern* with reference to the nucleus predetermines the future long axis of the sperm. This is further settled by the position that the centrosome takes up in the transforming spermatid. While the process of condensation of the spermatid mitochondria takes place the centrosome migrates to that part of the cytoplasm opposite the region of interruption of the chromatin lining of the nucleus (Fig. 40). Thus it comes to lie in between the nucleus on the one side and the *nebenkern* on the other (Fig. 41). While this polarity of the cell is being established, part of the Golgi bodies of the spermatid fuse together to form a structure which has the shape of an individual Golgi body. It has an outer incomplete chromophilic ring and an inner chromophobic area. This fused product of the Golgi bodies comes to be known as the acroblast (Fig. 42) and gives rise later to the acrosome of the mature sperm.

The components of the transforming spermatid thus consist of a nucleus which has chromatin inside in the form of a thin lining interrupted

at one region besides a few scattered granules in the centre, a *nebenkern* which lies opposite the interrupted region of the nuclear chromatin lining and which is differentiated into an outer chromophobic area and an inner dense homogeneous chromophilic zone, and a centrosome which lies as a minute densely staining granule between the nucleus and the *nebenkern*. Part of the Golgi bodies are present in the spermatid as a fused product known as the acroblast which has the structure of an individual Golgi body and is situated on one side of the cell occupying a position somewhat between the nucleus and the *nebenkern*. The rest of the Golgi bodies occur scattered in the cytoplasm (Fig. 42).

With the further transformation of the spermatid, the acroblast which is situated with the incomplete region of the chromophilic ring directed towards the nucleus, gradually approaches the nuclear membrane. In connection with the chromophobic area of the acroblast a vesicle—the acrosomal vesicle—is gradually developed (Fig. 43) within which a granule—the acrosomal granule—is soon elaborated (Fig. 44). The acroblast thus, due apparently to its secretory activity, elaborates a duplex product—the acrosomal vesicle and the contained granule. This complex structure—the acroblast with its products of secretion soon gets attached to the nuclear wall and once this connection is established it begins to travel along the nuclear wall to the anterior extremity of the nucleus (Fig. 45) and round the nucleus, to its posterior extremity (Fig. 46). When it has gained this position the acroblast deposits its duplex product of secretion—the acrosomal vesicle with the enclosed granule—on the nuclear wall after which the acroblast, now known as the Golgi remnant, recedes from its original position and travels towards the posterior end of the elongating spermatid (Fig. 47). Along with the progress of the acroblast and its products of secretion along the nuclear wall a gradual enlargement of the acrosomal granule within its vesicle takes place (Figs. 45 and 46) until finally when the duplex product of secretion is about to be deposited on the nuclear wall the granule occupies almost the whole of the interior of the vesicle. This acrosomal vesicle with the enclosed granule, which is hence forward collectively known as the acrosome, now begins its journey along the nuclear wall to that part of the nucleus which is destined to form the anterior end of the mature sperm (Fig. 47). Cross-sections of cysts containing spermatids at this period of development show that that part of the Golgi material that has not contributed to the formation of the acroblast has been gradually passed out of the cells and occurs as black masses in the central space of the cyst.



Figs 42-54—Fig 42 Spermatid Formation of acroblast Champy and iron haematoxylin $\times 2000$ Fig 43 Spermatid Formation of acrosomal vesicle Champy and iron haematoxylin $\times 2000$ Fig 44 Spermatid Formation of acrosomal granule within the acrosomal vesicle Champy and iron haematoxylin $\times 2000$ Fig 45 Spermatid Journey of acroblast along the nuclear wall to its anterior extremity Centrosome divided into proximal and distal centrosomes, the distal having migrated to the posterior end of the elongating nebenkern Champy and iron haematoxylin $\times 2000$ Fig 46 Anterior region of spermatid Journey of acroblast to the posterior limit of the nucleus. Golgi bodies receding to the posterior end of spermatid Champy and iron haematoxylin $\times 2000$ Fig 47 Anterior region of spermatid Acrosome deposited on the nuclear wall Golgi remnant receding to the posterior end of cell Champy and iron haematoxylin $\times 2000$ Fig 48 Spermatid Division of centrosome into two Flemming without acetic and iron haematoxylin $\times 2000$ Fig 49 Spermatid Orientation of proximal and distal centrosomes and division of the nebenkern Flemming without acetic and iron haematoxylin $\times 2000$ Fig 50 Anterior region of advanced spermatid Elaboration of the central granules in mitochondrial sheath Ludford $\times 2000$ Fig 51 Cross-section of the tail region of advanced spermatid Central granules present Champy and iron haematoxylin $\times 2000$ Fig 52 Anterior region of very advanced spermatid Nucleus triangular Acrosome distinct Champy and iron haematoxylin Diagrammatic Fig. 53 Anterior region

of very advanced spermatid Nucleus oval Acrosome at anterior end Champy and iron haematoxylin Diagrammatic Fig 54 Diagrammatic figure showing parts of sperm Chromatin rod in the centre of the nuclear head Acrosome distinct Axial filament in the region of the tail Champy and iron haematoxylin

N.B.—Figures 1 to 54 have been reduced to half the magnifications given

As these changes are going on, the centrosome which is situated in between the nucleus and the nebenkern divides into two (Fig 48), and from one of the two products of the divided centrosome the axial filament takes its origin. This is the proximal centrosome. The other gradually shifts away from its original position and wanders to the distal end of the nebenkern and there places itself close behind the division plane of the nebenkern (Fig 49). Soon after the division of the centrosome the nebenkern tends to present a bipartite appearance (Fig 49) the plane of division not always running through the exact centre of the mitochondrial mass. On closer observation the axial filament, which has by now grown out of the proximal centrosome, is seen to traverse the plane of division of the nebenkern and pass to the distal centrosome thereafter extending as a thin delicate flagellum in the substance of the cytoplasm of the spermatid (Figs 45 and 49).

Simultaneous with these changes the nucleus steadily decreases in size, the chromatin within the nucleus getting reduced to a very thin layer lining the nuclear wall. A few scattered chromatin granules lie in the interior of the nuclear cavity. With the progressive contraction of the nucleus, the nebenkern elongates considerably, the two respective halves sometimes elongating at a slightly varying pace. The elongating nebenkern thus forms a complete envelope for the growing axial filament to a certain extent of its length, the axial filament growing at a much quicker rate than the two respective halves of the nebenkern. A study of the later activities of the distal centrosome was not possible as it was difficult of recognition at the later stages of spermatid development.

During the initial period of formation of the nebenkern in the spermatid the cells are irregularly scattered within the cavity of the cyst but as further development of the spermatid proceeds they get arranged within the cyst with their anterior or nuclear ends directed towards the periphery of the cyst while their tail ends lie directed towards the centre. This peripheral arrangement of the cells in a cyst is characteristic of advanced spermatids. In haematoxylin preparations the nebenkern at this stage stains differently in different cells, some cells staining light, others dark, while still others show the component halves of the nebenkern coloured differently. This difference in colouration of the nebenkern halves has been frequently observed by previous authors but the phenomenon has so far remained unexplained.

When this stage of sperm formation, wherein the nebenkern halves have lengthened to an enormous extent is reached, a gradual dissolution of the chromophilic centre of the nebenkern is noticed, and as this process continues a new substance in the form of small oval granules develops within the outer chromophobic area. These are the central granules. The appearance of these central granules has been previously described by a few authors. Along with their formation there occurs a synchronous disappearance of the chromophilic substance of the nebenkern. This process of probable transformation of the chromophilic substance into the central granules continues until total disappearance of the chromophilic substance of the nebenkern takes place and as this process continues the central granules fuse together to form chains of granules which exist as such within the chromophobic area of the nebenkern (Fig. 50). Sections cut across the mitochondrial region of such spermatids exhibit a densely staining axial filament in the centre with the mitochondrial portion enclosing the central granules forming a complete sheath around it (Fig. 51). The mitochondrial halves meanwhile, due to their enormous lengthening, get twisted about each other with the result that the posterior region of the elongating spermatid presents a spiral appearance. As the mitochondrial sheaths get thus spun out and twisted certain bleb-like swellings appear along their course. These, however, in later stages of sperm formation become smaller and eventually disappear. Within these bleb-like swellings may be observed the central granules and though actual casting off of the mitochondrial substance has not been observed the disappearance of the bleb-like swellings from the region of the tail seems to suggest that part of the mitochondrial substance in the form of central granules is extruded from the growing spermatid.

The nucleus has by now become considerably smaller in size, it being reduced to about 1/8th that of the original spermatid nucleus. It presents a homogeneous appearance with just one deeply staining chromatin granule in the centre besides the lining of chromatin to the nuclear wall.

As development proceeds the spherical nucleus first assumes a conical shape (Fig. 52) but later it becomes oval (Fig. 53) and then elongates considerably until the chromatin lining of the nuclear wall gets condensed into a single chromatin rod lying along the central axis of the head (Fig. 54). The mitochondrial halves have by now elongated considerably along the tail the axial filament protruding beyond the posterior extremity of the mitochondrial sheath. The formation of the acrosome has also been completed and the Golgi remnant that has been cast off proceeds rapidly along the tail and along with other obviously useless products of spermiogenesis undergoes an apparent atrophy. These useless products of spermiogenesis are eventually

cast out of the cell in the form of protoplasmic balls which occur in groups in the region of the cyst where the sperm tails come to lie. In Mann-Kopsch preparations, the Golgi elements which form part of the debris of spermiogenesis occur as small masses in the region of the sperm tails. The final destruction of these waste products has not been observed but probably they serve as nourishment for the cells of the follicular epithelium.

The sperms at this time are arranged in regular compact bundles with their head ends directed towards the opening into the vas deferens each bundle being enclosed within a cyst, the walls of which are lined by a single layer of epithelial cells containing enormous nuclei. It is the cytoplasm of these epithelial cells that probably ingest the protoplasmic remains of the spermatids for the debris of spermiogenesis are not seen to occur in the ducts of the reproductive system.

The sperm heads from now on stain an intense and uniform colour and ordinarily no distinction can be made between the acrosome and the nucleus though careful destaining sometimes reveals the component parts distinctly. The mitochondrial region of the sperm however stains much lighter than the nuclear part. The sperms, when fully formed, are spirally twisted and become grouped in very compact bundles so that it is impossible to trace the length of a sperm from one end of it to the other. In this condition the sperms pass into the vas deferens.

THE SPERMATOZON

The mature sperm consists essentially of a head composed of a solid, homogeneous core of chromatin to which is attached, projecting anteriorly, the acrosome, a long cylindrical tail containing the axial filament in the centre and surrounded by the mitochondrial sheath, and lastly, the proximal and distal centrosomes, the proximal being interposed between the nuclear head and the mitochondrial tail, the distal situated probably at the posterior end of the mitochondrial sheath.

THE SPERMATOZOA IN THE DEFERENT DUCTS

Within the vas deferens the sperm clumps come together and from now on the identity of each individual bundle is lost, the sperms occurring hopelessly twisted about. The mature sperms travel down the deferent ducts and are stored in the dilated portions of the vas deferens for a time, the seminal vesicles in this animal not serving as storage organs and appearing as sterile and non-functional appendages of the deferent ducts. Cross sections of the seminal vesicles do not show the presence of sperms. During copulation, the sperms are passed to the ejaculatory duct from whence they are transferred to the genital aperture of the female.

DISCUSSION

(a) *The Idiochromosomes*

From observations on the Hemiptera, Wilson has drawn five types of chromosome groups. Taking these in order the first type is characterised by both the sexes possessing a pair of equal idiochromosomes of which *Oncopeltus* is an example. *Nezara* also was first included under this group though later it was found necessary to place it under the second group which is characterised by both the sexes possessing the same number of chromosomes, the female possessing a pair of equal idiochromosomes, the male a pair of unequal idiochromosomes, the result being that half the spermatozoa receive the large idiochromosome and half the small, e.g., *Euschistus*, *Cerus*, *Podisus*, *Lygaeus*, etc. The third group is distinguished by the female chromosome groups having one more chromosome than the male. The male here has an unpaired idiochromosome and an odd spermatogonial number, half the spermatozoa receiving the idiochromosome, the other half being without it, the female with an equal pair of idiochromosomes like the unpaired one of the male, e.g., *Pyrrhocoris*, *Largus*, *Alydus*, *Harmostes*, *Protenor*, *Anasa*, etc. The fourth group is distinguished by the female groups having two more chromosomes than the male, the male possessing a pair of unequal idiochromosomes, half the spermatozoa receiving both these chromosomes and hence two more than the other half. This type characterises the form *Syromastes*. The last group, of which *Galgulus* is an example, is characterised by the female groups having three more chromosomes than the males, half the spermatozoa receiving three more chromosomes than the other half.

In *Laccotrephes* it will be observed that the type of chromosome combination is similar to that described by Wilson under the second category of chromosome groups characteristic of *Euschistus* and others, the male possessing a pair of idiochromosomes that are unequal in size, dividing equationally in the first division and reductionally in the second, unlike the autosomes where the reverse is the order, the result being the production of two kinds of spermatids, one with half the number of spermatogonial autosomes plus the large idiochromosome and the other with half the number of spermatogonial autosomes plus the small idiochromosome.

The idiochromosomes in the spermatogonial metaphase plate do not show much difference in size though they may be observed as two deeply-staining chromosomes and situated somewhere in the centre of the chromosomal group and never anywhere on the periphery.

During the period of growth of the primary spermatocyte the idiochromosomes become distinctly visible lying at first separate but later coming

together and occupying a position in close contact with each other and with the nuclear membrane. A preliminary conjugation between the two idiochromosomes now takes place and in this respect *Laccotrephes* agrees with the forms *Brochymena* and *Euschistus* unlike *Lygaeus* and *Cenetus* wherein no such phenomenon occurs

In the early stages of the growth period the larger idiochromosome alone gets elongated in form, but in the later stages an elongation of the smaller idiochromosome as well takes place the relative size between the two however being still maintained. It may be mentioned, that this difference in size between the idiochromosomes during the period of growth of the primary spermatocyte has been previously observed by Montgomery in forms like *Trichopepla*, *Peribalus* and *Euschistus tristigmus*.

Within the metaphase plate of the primary spermatocyte no arrangement of the idiochromosomes within an autosomal ring as occurs in *Lygaeus* is observed, the idiochromosomes however occurring somewhere near the centre of the equatorial plate. In *Cenetus* and *Euschistus* the larger idiochromosome is described to frequently occupy a place in the outer ring. In a side view of the spindle the chromosomes exhibit a symmetrical dumbbell-shape and when division occurs they are equally divided with the result that two exactly similar daughter groups of twenty-two chromosomes (twenty autosomes and two idiochromosomes) each are formed. The rate at which the idiochromosomes travel to the two poles does not vary with that of the autosomes though such a difference in rate has been observed in *Lygaeus* by Wilson (1905).

Immediately after division, the idiochromosomes still lie free of each other paralleling *Lygaeus* in this respect but differing from *Cenetus* where they sometimes lie in close contact with each other already forming an unequal dyad. In *Laccotrephes* the formation of the dyad takes place at a slightly later stage, that is, just before the resolution of the spindle, the dyad presenting itself as a deeply staining structure within the group of chromosomes. In the equator of the secondary spermatocyte therefore the idiochromosomes appear as an unequal bivalent with the result that with the ensuing division half the products of division of the secondary spermatocyte receives twenty autosomes and the small idiochromosome while the other half receives twenty autosomes and the large idiochromosome.

In the process of division of the secondary spermatocyte the idiochromosome-conjugants separate from each other prior to the autosomes, the former leading the way of the latter along the spindle fibres to the two poles. The presence of the idiochromosome dyad in the secondary spermatocyte

is recognizable by their unequal size, deeply staining capacity, position on the metaphase plate and finally, separation of its component chromosomes prior to the division of the autosomes

The division of the secondary spermatocyte is therefore equational for the autosomes and reductional for the idiochromosomes this process being the reverse of what occurs in the primary spermatocyte division. In this respect *Laccotrephes* agrees with the forms *Cenus*, *Lygaeus*, *Brochymena*, *Euschistus* and *Podisus* and the type of chromosome combination conforms to the second of the five types of chromosome groups met with in the Hemiptera and described by Wilson (1909) in the fourth series of his "Studies on Chromosomes"

(b) *The Mitochondria*

(1) *The Origin of the Mitochondria* —The question of the origin and increase in number of the mitochondria has been the subject-matter for considerable controversy in recent years. Four views have been postulated regarding this question. The first according to Duesberg and others, stresses that no *dé novo* origin of the mitochondria takes place but that by a continual process of division of the pre-existing ones new mitochondria are produced. This is known as the "genetic continuity hypothesis" and has been favoured by Bowen (1920), Wilson (1925) and Chickering (1927). Opposed to this is the second hypothesis developed by Goldschmidt (1909), Buchner (1909) and others according to which the mitochondria have a nuclear origin and thereby known as the "chromidial hypothesis". A third hypothesis, postulated by Vejdovsky (1907 and 1912) derives the mitochondria as cytoplasmic structures having their origin in the "regressive modification" of the sphere material. A fourth view as put forth by Shaffer in 1920 holds that "the mitochondria arise as differentiated parts of the cytoplasm through specific chemical (enzymes) reactions of the nucleus upon the products of assimilation of the cell". This is the "interaction theory". Montgomery (1911) and Browne (1913) also expressed their views that the mitochondria may result by the interaction between nucleus and cytoplasm.

In *Laccotrephes griseus*, mitochondria in the youngest germ cell—the spermatogonia—occur as a polar cap to the nucleus. This occurrence of a polar cap of mitochondria has been frequently described in many Hemiptera by various authors. That the mitochondria in the earliest germ cells should occur in such close relationship with the nucleus suggests in itself that the nucleus is partly responsible for the initial elaboration of the mitochondria. And the fact that the chromatin in the nucleus and the mitochondria in the cytoplasm are of very different appearance and chemical behaviour further

suggests that the mitochondria may be formed in the cytoplasm as a result of chemical reactions of the nucleus on certain cytoplasmic inclusions of the cell, the inclusions probably being of the nature of products of assimilation in the cell

(ii) *The Chondroconts*—The transformation of the mitochondria from the granular type—the chondriosome, to the filamentous type—the chondrocont, has been frequently described by many authors. Among the Hemiptera, Browne (1913) described the process in *Notonecta*, Shaffer (1920) in the spermatocyte of *Cicada*, Bowen (1920) in *Euschistus*, Payne (1927) in *Gelastocoris* and Pollister (1930) in *Gerris*. While the existence of the filamentous form of mitochondria has been described by various contributors, opinions differ as to the exact method of formation of this mitochondrial element. Shaffer concluded in 1920 that the mitochondrial filaments in *Cicada* arise as a result of the linear fusion of separate granules and so also Chickering in 1927 from his observations on the Belostomatidae. Bowen from his studies on the Pentatomidae found the granular chondrosomes becoming thread-like but was uncertain as to the exact method by which the change was effected. Payne and Pollister contribute to the view that the transformation of the granular chondrosomes to the filamentous type takes place by a direct elongation of the granular mitochondria and not by a process of linear fusion of the individual granules.

In the spermatogonial cells of *Laccotrephes griseus* mitochondria occur in the form of a finely granular crescentic mass applied close to the nuclear wall. In later stages this cap gets broken up and the mitochondria occur as short filaments—the chondroconts. This form of mitochondria exists throughout the spermatocyte stages until in the spermatid, the spherical Nebenkern is constituted. In the form studied no stage in which the mitochondria present a vesicular form has been observed. It is possible to imagine that a linear fusion of the granules could constitute the elongated form of mitochondria as present in the spermatocyte cells of the form studied and as has been described by other authors in forms like *Cicada* (Shaffer, 1920) and those of the Belostomatidae (Chickering, 1927). It is likewise possible to derive the elongated vesicular form of mitochondria as described by Payne (1927) in *Gelastocoris*, from rings previously formed by the thread-like mitochondria of the growth period. In the latter case however preliminary fusion of the granular mitochondria in a linear fashion seems to be essential for the later development of the elongated vesicular forms though this essential process was not described as such by Payne in his observations on the form. Browne (1913) describes in *Notonecta*, that the filamentous

forms of mitochondria are directly derived from the vesicular forms by a disappearance of the surrounding substance In regard to Browne's work on *Notonecta* it may be suggested that the spherical forms may have been initially formed by a circular grouping of the granular mitochondria These on breaking up in the later stages may give rise to the filamentous form described In either of the two last mentioned cases however it may seem necessary that the rings as in *Gelastocoris* or the vesicles as in *Notonecta*, are first formed by a fusion of the individual granules from which the elongated form may be subsequently derived

It may therefore be concluded that a side-by-side fusion of the granular mitochondria is an essential factor for the formation of either straight rods or spheres, the latter breaking up subsequently to form thread-like mitochondria or getting drawn out to form elongated vesicles

Browne, in her observations, probably overlooked the actual process of initial formation of the vesicular form of mitochondria from the granular and arrived at the conclusion that they arise by the swelling up of each individual mitochondrion and not by a fusion of the granules in a spherical manner Payne's observations on *Gelastocoris* help to elucidate the position, for the formation of rings here is clearly depicted in his figures of the growth period That the thread-like mitochondria formed in the earlier stages by the breaking up of the mitochondrial cap in the spermatogonia, are products formed by the linear fusion of the mitochondrial granules and not by the elongation of the individual granules is probably proved by the fact that the filamentous mitochondria formed are not seen to be all of the same size and form If the mitochondrial cap, which is formed of uniform granules of mitochondria, should get disrupted and each elongate into a filamentous mitochondrion we should expect all the filaments to be almost of the same size and form No cell ever exhibits a condition as such and this fact in itself affords enough strength for one to conclude that the filamentous mitochondria are produced not by an elongation of the individual granules but by a gradual linear fusion of the existing granular forms

The observations of Pollister on *Gerris* now remain to be considered Here, the spermatogonial cell, as usual, exhibits a polar cap of mitochondria which in the later growth stages occur as elongated filaments of unequal length Towards the completion of the growth period the filaments greatly increase in diameter and in a slightly later stage some of them break up into spheres while others remain passive The unusual size of the filaments and their extraordinary behaviour in breaking up into spheres in the later stages are extremely remarkable That minute granules of mitochondria should

reach such enormous dimensions seems, in the first place, to be inconceivable while, in the second, the irregular mode of division of only some of the chondroconts seems to be unlike the behaviour that would be expected if individual granules were transformed into filaments. It therefore leads one to conclude from the size and behaviour of the chondroconts, that the origin of the chondroconts could only lie in the fusion of the minute grains of mitochondria or chondriosomes and never in the elongation of each individual granular chondriosome.

Before one arrives at a conclusion it is perhaps best to cite the example of *Centrurus* (Wilson, 1916 and 1925) in which the distribution of mitochondria is effected by a preliminary fusion of the granular mitochondria to form a distinct open ring, which in the later division stages divides into four equal masses which are distributed to the daughter cells, each spermatid consequently receiving the exact fourth of the entire ring. Here the direct fusion of the mitochondria to form a ring is clearly depicted, and though *Centrurus* falls out of the region of Hemipteran spermatogenesis, may not one gather from such observations that a general tendency to such fusion does exist in the granules, and that this is exhibited in certain forms by the presence of filamentous mitochondria in their cells? In others where such a tendency to form chondroconts is absent the granules fuse and then swell into vesicles, which later contribute to the formation of the spherical nebenkern in the spermatid.

(iii) *The origin of the central granules*—The presence of the central granules within the mitochondrial sheaths of the developing spermatids evokes the question of their origin. Holmgren (1902) and Vejdovsky (1912) studied this question in previous years, the former basing his observations on *Silpha* and the latter on *Diastrammena*. Holmgren thought that the beaded thread (central granules) arose from the central chromophilic mass by a process of splitting off while Vejdovsky believed that the process was a transformation of the "Chondriom" (chromophilic substance) into the granular components ("Mitochondrien") from which it originally arose.

From observations on the spermatid cells and transforming spermatids of *Laccotrephes* it seems likely that the view that the origin of the central granules lies in the chromophilic region of the elongating nebenkern, might be adhered to, for with the dissolution of the latter the gradual evolution of the former is observed. Bowen (1922b) seems to adhere to the same view as that put forth by previous authors and also ascribes to the central substance the source of the bleb-like swellings later developed on the tail sheaths.

SUMMARY

- 1 The male reproductive system is described
- 2 There are primary and secondary spermatogonia the latter being distinguished from the former by their radial arrangement in cysts Within the spermatogonium the mitochondria occur as a conical cap applied close to the nuclear wall while the Golgi bodies exist scattered in the cytoplasm A centrosome is present
3. During mitosis the Golgi and the mitochondria get approximately distributed to the two daughter cells The diploid number of chromosomes is forty-two, the form possessing an XY pair of chromosomes
- 4 Nutritive cells are present in the spermatogonial region of the testis
- 5 The primary spermatocyte does not undergo a period of rest The nucleus quickly emerges into its prophase and passes through all the stages from the leptotene to the tetrad stage The idiochromosomes first remain condensed and independent of each other but later they come together, elongate, fuse and then separate and each attains a dumbbell-shaped appearance.
- 6 The mitochondrial granules fuse together to form filaments—chondriocysts—and are scattered irregularly in the cytoplasm The Golgi bodies occur as deep crescents irregularly distributed in the cytoplasm The centrosome is distinct
- 7 A polar view of the primary spermatocyte shows twenty-two chromosomes—twenty autosomes plus two idiochromosomes The division hence is reductional for the autosomes and equational for the idiochromosomes The Golgi and mitochondria are distributed approximately to the two secondary spermatocytes
- 8 The secondary spermatocyte does not undergo a period of rest The idiochromosomes pair to form an unequal dyad on the spindle of the secondary spermatocyte. A polar view of the secondary spermatocyte reveals the haploid number—twenty one—of chromosomes Division here is therefore equational for the autosomes and reductional for the idiochromosomes, the components of the idiochromosome dyad separating from each other on the spindle prior to the division of the autosomes
- 9 The spermatid is a very small cell, the chromatin at first forming an irregular mass within the interior of the nucleus but later getting distributed in such a way as to form an interrupted band of lining to the nuclear wall. Within the cytoplasm a centrosome, mitochondria in the form of a spherical nebenkern and a few scattered Golgi bodies exist

10 The nebenkern at first exhibits a vacuolated appearance but later gets differentiated into an outer homogeneous chromophobic area and an inner, spherical homogeneous chromophilic zone, the nebenkern itself lying opposite the interrupted region of the chromatin lining of the nucleus. Part of the Golgi bodies fuse together to form an acroblast and lies on one side of the cell in between the nucleus and the nebenkern. The rest of the Golgi degenerate and are finally expelled from the cell. The centrosome migrates towards the nucleus and comes to lie in between the nucleus and the nebenkern.

11 With the transformation of the spermatid the centrosome divides and from one of its products the axial filament grows. This is the proximal centrosome. The distal centrosome migrates to the posterior limit of the nebenkern. The axial filament thus divides the nebenkern into two halves which elongate considerably. The axial filament grows at a quicker rate than the mitochondrial halves and even extends further than the posterior extremity of the cell.

12 In connection with the chromophobic part of the acroblast a vesicle is formed within which a granule is soon elaborated. This duplex formation of the acroblast—the acrosomal vesicle with the enclosed granule—is deposited at the posterior extremity of the nucleus after a preliminary journey of the acroblast along the nuclear wall. This duplex product of the acroblast is henceforward known as the acrosome.

13 The acrosome travels towards the anterior limit of the nucleus while the acroblast—henceforth known as the Golgi remnant—travels towards the posterior end of the elongating spermatid. The spermatids at this stage are arranged radially within the cyst the head ends of the spermatids directed towards the periphery of the cyst while the tail ends point towards the centre.

14 The nucleus steadily decreases in size and the spherical nucleus finally attains an elongated appearance, the chromatin lining within it getting approximated to each other to form a single chromatic rod in the centre.

15 The mitochondrial halves elongate to a considerable extent and get spirally twisted about each other. Meanwhile the appearance of the central granules within the chromophobic area is seen along with a dissolution of the chromophilic interior. A little later the formation of the bleb-like swellings is observed in the tail region of the elongating spermatids which disappear with the further development of the spermatid.

16. The acrosome has by now reached the anterior extremity of the nucleus and formed the definitive acrosome. The Golgi remnant is expelled from the spermatid.

17. When the formation of the sperm is nearing completion all the useless products of spermiogenesis are cast out of the sperm in the form of protoplasmic balls into the central cavity of the cyst. These protoplasmic balls are probably ingested by the epithelial cells of the cyst wall.

18. The fully formed sperm has an elongated nuclear head to the anterior extremity of which the acrosome is attached, a long cylindrical mitochondrial tail between the component halves of which the elongated axial filament is lodged, and a proximal and distal centrosome, the proximal being situated in between the head and the tail and the distal probably at the posterior end of the mitochondrial sheath.

19. The sperms when fully formed are massed into bundles within the cysts and stored for a time in the caudal regions of the vas deferens before they are finally transferred to the genital aperture of the female.

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ABBREVIATIONS

<i>a</i>	acrosome	<i>g r</i>	golgi remnant
<i>ab</i>	acroblast	<i>m c</i>	mitochondrial cap
<i>a f</i>	axial filament	<i>m f</i>	mitochondrial filament
<i>a g</i>	acrosomal granule	<i>m g</i>	mitochondrial granule
<i>a v</i>	acrosomal vesicle	<i>m.s</i>	mitochondrial sheath
<i>a v.d</i>	ampulla of vas deferens	<i>n</i>	nucleus
<i>a v.e</i>	ampulla of vas efferens	<i>ne</i>	nebenkern
<i>c</i>	centrosome	<i>nu</i>	nucleolus
<i>c₁</i>	proximal centrosome	<i>p s p m p</i>	primary spermatocyte metaphase plate
<i>c₂</i>	distal centrosome	<i>p s p m s</i>	primary spermatocyte metaphase spindle
<i>c g</i>	central granule	<i>pl</i>	plasmosome
<i>c w</i>	cyst wall	<i>s k</i>	synthetic knot
<i>cd v d</i>	caudal region of vas deferens	<i>s sg</i>	secondary spermatogonium
<i>ch</i>	chromosome	<i>s sp m p</i>	secondary spermatocyte metaphase plate
<i>ch g</i>	chromatin granule	<i>s sp m s</i>	secondary spermatocyte metaphase spindle
<i>ch m</i>	chromatin mass	<i>sv</i>	seminal vesicle
<i>ch r</i>	chromatin rod	<i>t f</i>	testicular follicle
<i>chb r ne</i>	chromophobic region of neben-kern	<i>tet</i>	tetrad
<i>chp r ne</i>	chromophilic region of neben-kern	<i>v e</i>	vas efferens
<i>cr v.d</i>	cranial region of vas deferens	<i>x.ch</i>	X-chromosome
<i>e d</i>	ejaculatory duct	<i>xy.ch</i>	XY-pair of chromosomes
<i>g</i>	golgi	<i>y.ch</i>	Y-chromosome
<i>g a</i>	genital aperture		
<i>g m</i>	golgi mass		

CYTOTOLOGICAL STUDIES IN BIGNONIACEÆ

Part IV. The Cytology of *Dolichandrone Rheedii* Seem and Allied Genera

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1. INTRODUCTION

THIS is a continuation of the author's work (Venkatasubban, 1944) on the cytology of the family Bignoniaceæ. In this study, the somatic and meiotic chromosome numbers, together with such details as secondary association and nature of the anther tapetum, have been worked out for some of the hitherto uninvestigated species of this family. An appendix containing a complete list of chromosome numbers for the different members of this family, investigated so far, is also included.

2. MATERIALS AND METHODS

Most of the materials for the present study were obtained in the form of seedlings from the Peradeniya Botanic Gardens through the courtesy of the Curator, Mr. T. H. Parsons, to whom my grateful thanks are due.

Root tips and anthers were first given a prefixation in Carnoy's fluid (3 parts of absolute alcohol, 2 parts of chloroform and 1 part of glacial acetic acid) for about 50 seconds before they were transferred to and left in Navaschin's fluid for a period of 24 hours. After the lapse of the first 3 or 4 hours, when the colour of the Navaschin's solution became somewhat dark, it was replaced by a fresh quantity of the fixative. In the case of the root tips, the time of division in the different materials was somewhat erratic. However, in most cases suitable plates were obtained by fixing the tips between 10.30 A.M., and 12 Noon on warm sunny days. For the study of meiosis, the anthers were first subjected to a preliminary examination under aceto-carmine to determine whether they were at the right stage of division. Then, they were sliced into thin pieces before treating them with Carnoy's and Navaschin's fixatives. A thorough washing in tepid water, extending for a period of two hours and involving several changes, was found necessary as otherwise, the sections especially those of anthers, took a yellowish tinge owing to the presence of some undissolved chromic acid in the cell walls. Dehydration was effected gradually by substituting various grades of ethyl alcohol. Up to the 70% alcohol stage, the materials were allowed to remain in each grade for only a couple of hours as this reduced the possibility of their getting macerated due to the action of very dilute alcohol. Chloroform was used as a solvent for paraffin. After embedding, the root tips and anthers were sectioned at a thickness of 14 to 16 microns and stained exclusively in Newton's Iodine-Gentian violet. The drawings were made at table level with a camera lucida employing a Zeiss flourite objective $\times 100$ and oculars $\times 30$ or $\times 20$.

3 OBSERVATIONS

(a) Somatic Chromosomes—

The somatic chromosome numbers have been determined, for the first time, for the following species.—

<i>Bignonia Unguis-cati</i> Linn.	..	$2n = 40$
<i>Heterophragma adenophyllum</i> Seem.	..	$2n = 40$
<i>Markhamia platycalyx</i> Sprague	..	$2n = 40$
<i>Markhamia hildebrandtia</i>	..	$2n = 40$
<i>Stereospermum xylocarpum</i> Wight	..	$2n = 40$
<i>Stereospermum suaveolens</i> DC.	..	$2n = 40$
<i>Tabebuia spectabilis</i> Nichols	..	$2n = 40$
<i>Spathodea nilotica</i> Seem	..	$2n = 26$

Bignonia Unguis-cati—Forty rod-shaped somatic chromosomes were counted in the root tips of the plant examined (Text-fig. 1). Almost all of

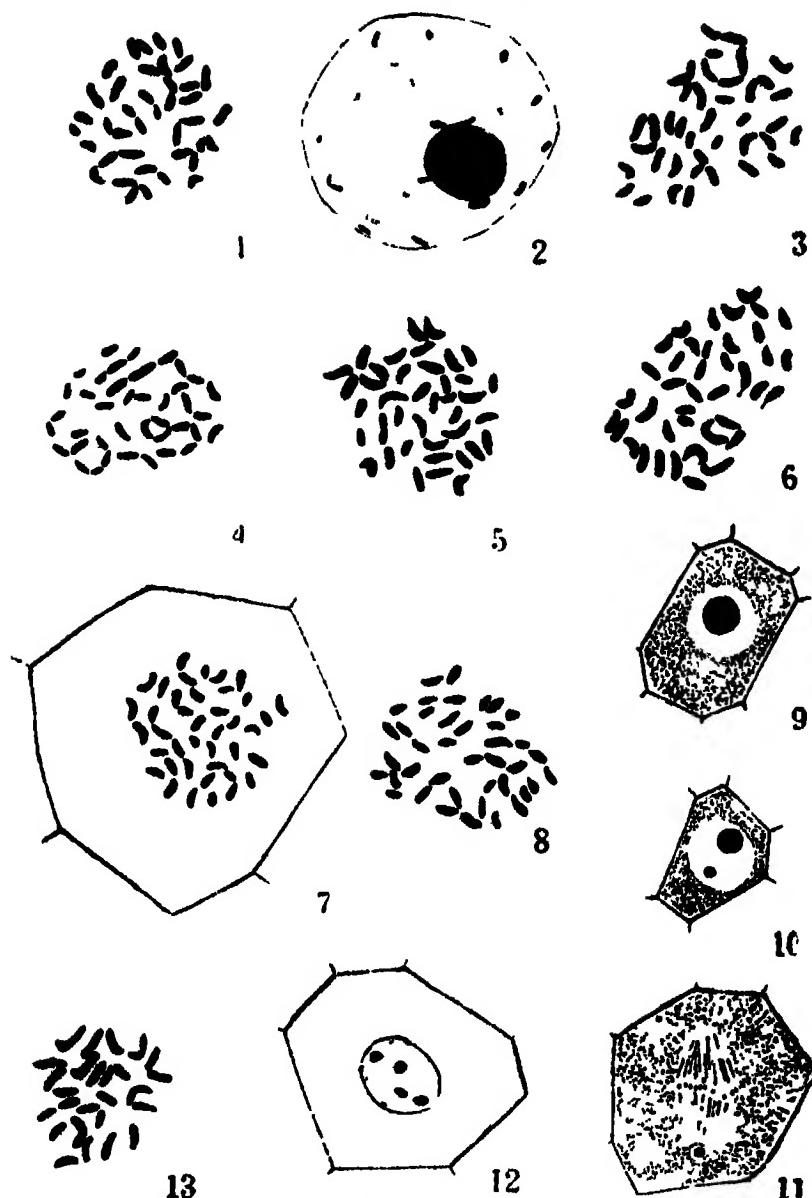
them were alike in their configuration; but four to six were somewhat smaller than the rest. Each chromosome exhibited only one constriction which was either sub-median or sub-terminal. Prochromosomes were also observed (Text-fig. 2), but the exact number could not be determined owing to their peripheral distribution. Some of the cells exhibited a single prominent nucleolus and in some such cases (Text-fig. 2) four prochromosomes were found attached to it.

Heterophragma adenophyllum—This species also showed 40 rod-shaped chromosomes in the somatic cells of the root tip (Text-fig. 3). They were mostly similar not only to one another but also to those of *Bignonia Unguis-cati*.

Markhamia—Two species of *Markhamia* (*M. platycalyx* and *M. hildebrandtia*) were examined. In both cases the diploid number ($2n$) was found to be 40. The chromosomes of *M. platycalyx* (Text-fig. 4) were slightly thinner as compared to those of *M. hildebrandtia* (Text-fig. 5). Further, in *M. platycalyx*, the chromosomes were mostly rod-like while in *M. hildebrandtia*, they were found to be slightly crescent-shaped and somewhat bigger. In both the species, the chromosomes exhibited only one constriction of the sub-median or sub-terminal type.

Stereospermum—*Stereospermum suaveolens* as well as *S. xylocarpum* exhibited a diploid chromosome number of $2n = 40$. As in the previous cases, the chromosomes of *S. suaveolens* (Text-fig. 6) revealed either a sub-median or sub-terminal constriction. One pair of chromosomes was accompanied by satellites and some of the chromosomes were crescent-shaped. The somatic chromosomes of *S. xylocarpum* (Text-fig. 7) were slightly smaller than the other species. They were mostly short and rod-shaped.

Tabebuia spectabilis.—Text-fig. 8 represents the somatic chromosomes ($2n = 40$) of this species, which again bear a close resemblance to those of *Bignonia Unguis-cati* or *Stereospermum xylocarpum*. Most of the chromosomes appeared to possess a sub-terminal constriction. In the resting condition, the nucleus usually showed a single large nucleous; but sometimes, an additional smaller one was seen, which may be called the 'secondary nucleolus' (Text-fig. 9). Presumably the latter arose by a process of budding although this was not actually observed in any of my preparations. In a few cases it was found to persist even during mitosis. In Text-fig. 11, which represents an early anaphase stage, there can be seen a small nucleolus at the lower pole which is surrounded by a halo. From these, it may be inferred that the 'secondary nucleolus' does not participate in the reorganization of the chromosomes. A similar case of persistence of the nucleolus



Text-Figs. 1-13.—Figs 1-8 and 13 have been drawn at an approximate magnification of $\times 5400$, while Figs. 9-12 have been drawn $\times 1800$. Fig. 1. Somatic metaphase plate of *Bignonia Unguis-cati* ($2n = 40$). Fig. 2. Early prophase in the root-tip cell of *Bignonia Unguis-cati* showing 4 attached prochromosomes to the nucleolus. Fig. 3. Somatic metaphase plate of *Heterophragma adenophyllum* ($2n=40$). Figs 4 and 5. Somatic metaphase plates of *Markhamia platycalyx* and *M. hildebrandtii* ($2n = 40$). Figs 6 and 7. Somatic metaphase plates of *Stereospermum xylocarpum* and *S. suaveolens* ($2n=40$). Fig. 8. Somatic metaphase plate of *Talbotia*

spectabilis ($2n=40$) Fig. 9 A cell from the periblem region in the root-tip of *Tabebua spectabilis* showing a resting nucleus and a prominent nucleolus Fig. 10. Same as above but showing a secondary nucleolus Fig. 11. Early anaphase in the somatic cell of *Tabebua spectabilis* showing secondary nucleolus in the vicinity of a pole Fig. 12 Early prophase or late telophase in the root-tip cell of *Tabebua spectabilis* showing 4 nucleoli Fig. 13. Somatic metaphase plate of *Spathodea nilotica* ($2n=26$)

has been reported in *Kigelia pinnata* (Venkatasubban, 1944) The presence of prochromosomes, in the root tip cells, was also found to be a regular feature of this species At the early prophase of mitosis, 4 small nucleoli (Text-fig 12) were counted But these seem to fuse afterwards since in later stages only one prominent nucleolus is to be seen

Spathodea nilotica —The somatic chromosomes of this species (Text-fig 13) are quite distinct both in their morphology as well as their number from those of the rest of the species examined The diploid number for this species was found to be 26, the chromosomes are somewhat longer and thinner than in the other species and have a sub-median constriction

(b) Meiotic Chromosomes—

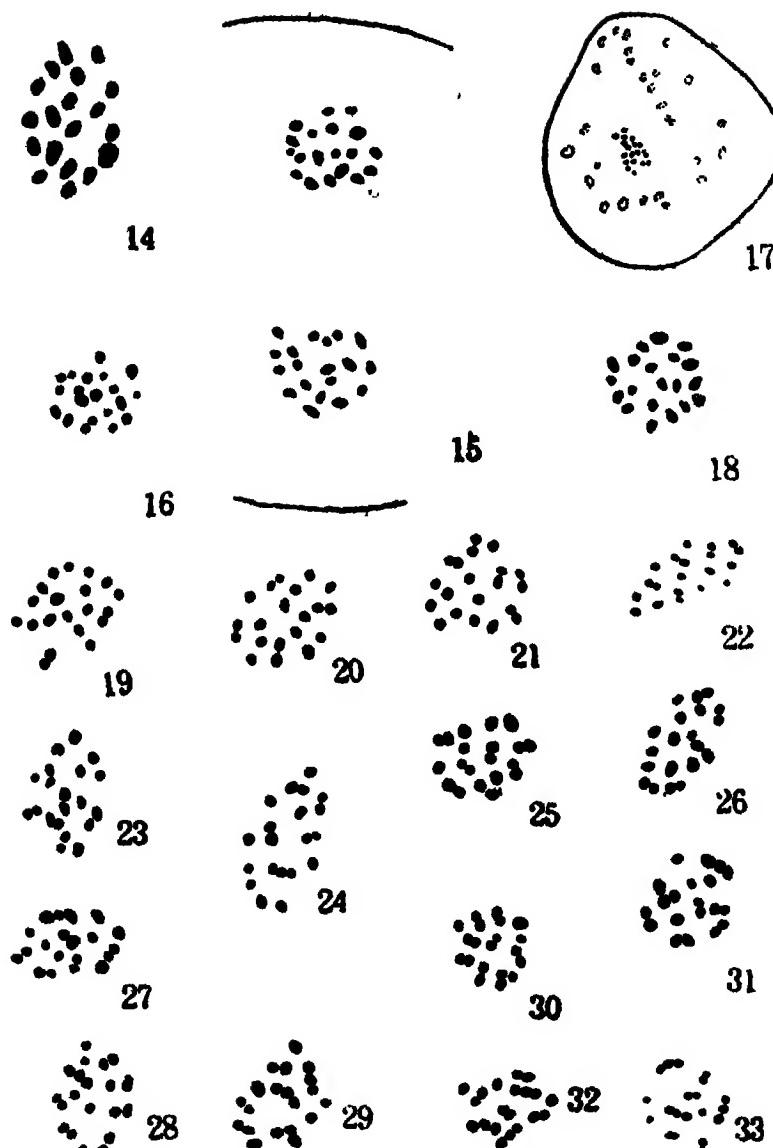
The haploid number was determined for the following species from counts made in the first as well as the second metaphase :—

<i>Tecoma serratifolia</i> Don		$n = 19$
<i>Parmentiera edulis</i> D C	..	$n = 20$
<i>Dolichandrone Rheedit</i> Seem		$n = 20$

Tecoma serratifolia —Stages earlier than the first metaphase were not studied In the first metaphase 19 bivalents (Text-fig 14) were counted Four of these were slightly bigger than the rest In the second metaphase the distribution was found to be 19/19 (Text-fig 15), showing thereby that a regular disjunction had taken place Some of the univalents appeared slightly bigger than the rest

Parmentiera edulis —In this species only the first metaphase plates were available and 20 gemini (Text-fig 16) were counted from a polar view of the first and heterotypic division This corroborates the previous determination of $2n = 40$ (Venkatasubban, 1944) from the root tip cells of the same species Some of the metaphase plates examined indicated a secondary association but this point could not be pursued further owing to want of more material

Dolichandrone Rheedit —The young flower buds of this species (before the opening of flowers) contain watery secretion between the calyx and the corolla, which is probably due to the activity of 'epidermal hydathodes' found lining the inner surface of the calyx and the outer apical region of the



Text-Figs. 14-18—Fig. 14 First metaphase plate in the P.M.C. of *Tecoma serratifolia* ($n=19$) $\times 3600$ Fig. 15 Second metaphase of *Tecoma serratifolia* showing the 19/19 distribution $\times 3600$ Fig. 16 First metaphase in *Parmentiera cerifera* ($n=20$). $\times 3600$ Fig. 17 A P.M.C. of *Dolichandrone Rheddi* in the first metaphase showing starch-like inclusions. $\times 1800$. Fig. 18 First metaphase in the P.M.C. of *Dolichandrone Rheddi* showing 20 gemini in an unassociated condition.

Text-Figs. 19-33 represent various degrees of secondary association in the first and the second metaphase stages. These have been drawn at an approximate magnification of 3600 diam.

Fig. 19. Shows an association of 16 (1) 2 (2). Fig. 20 Shows an association of 12 (1) 4 (2).
 Fig. 21. Shows an association of 10 (1) 5 (2) Fig. 22 Shows an association of 10 (1) 5 (2)
 in M. II Fig. 23 Shows an association of 8 (1) 6 (2) Fig. 24 Shows an association of
 9 (1) 4 (2), 1 (3) Fig. 25 Shows an association of 6 (1) 7 (2) Fig. 26 Shows an association
 of 8 (1) 3 (2) 2 (3) Fig. 27 Shows an association of 6 (1) 4 (2) 2 (3) Fig. 28 Shows an
 association of 5 (1) 6 (2) 1 (3) Fig. 29 Shows an association of 5 (1) 3 (2) 3 (3) Fig. 30
 Shows an association of 3 (1) 7 (2) 1 (3) Fig. 31 Shows an association of 4 (1) 5 (2) 2 (3)
 Fig. 32 Shows an association of 3 (1) 4 (2) 3 (3) Max association Fig. 33 Shows an associa-
 tion of 3 (1) 4 (2) 3 (3) Max association in M II

corolla Similar structures have been found in *Spathodea campanulata* and have already been described previously by Raghavan and Venkatasubban (1940) The pollen mother cells were densely packed with certain food bodies (Text-fig. 17) Hand sections treated with iodine revealed plenty of starch grains in the wall cells of the anther; but the food particles mentioned above in the pollen mother cells were only faintly stained Their exact nature is, therefore, rather doubtful * They stained readily with gentian violet and as such it was found to be a matter of considerable difficulty to differentiate them from the chromosomes. In the first metaphase (Text-fig. 18) 20 bivalents were counted and also a number of metaphase plates of the second division showed the expected 20/20 distribution. Both during the first and second metaphase stages the chromosomes revealed a secondary association which is described below

(c) Secondary association in *Dolichandrone Rheedii*—

Though some data have been obtained regarding secondary association in this species they are as yet rather incomplete; for, the various groupings were observed only in about 33 pollen mother cells studied. The chief difficulty in making further observations was due to the presence of the numerous food bodies around the metaphase plates which have been referred to above. Besides this, due to some reason or other, most of the preparations showed only a side view of the plates The different associations observed in the first and second metaphase plates are indicated in Table I.

The above groupings are illustrated in Text-figs 19-33. The most frequent among these are the ones showing an association of 8. Nearly one third of the pollen mother cells showed this association. Next in the order of frequency come those with an association of 10. Six different pollen mother cells gave evidence of this type. Of these, one set consisted of 3 (1), 4 (2) and 3 (3) while the other association was found to be 2 (1), 6 (2) and 2 (3) Of the two groupings, the former occurred in 5 cases while the latter

* Such starch-like inclusions were also found to be a regular feature of the pollen mother cells in *Spathodea campanulata* Beauv (Raghavan and Venkatasubban, 1940)

TABLE I

Number of secondary associations	Number of bivalents in association				No. of cases	Total
	1	2	3	4		
2	16	2			1	1
4	12	4			2	2
5	10	5			2+1	3
6	8	6			2	3
7	9	4	1		1	
	6	7			2	3
8	8	3	2		1	
	6	4	2		5+1	10
5	5	6	1		4	
9	5	3	3		1	
	3	7	1		1	4
10	4	5	2		2	
	3	4	3		4+1	6
11	2	6	2	1*	1	1
	1	6	1	1*		
						33

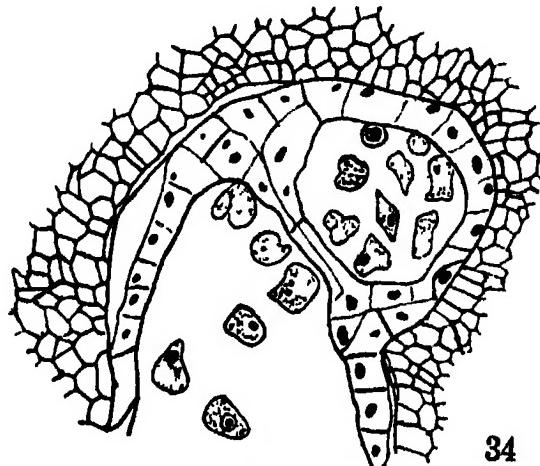
* An aberrant case

was seen only once. In one case an association of 11 was found but this must be regarded as abnormal since it exhibited a grouping of 4 bivalents which is rather uncommon. So leaving this out of consideration, the maximum association for this species has been found to be ten. The same value for maximum association has been independently arrived at for a number of other species like *Crescentia cujete*, *Tabebuia pentaphylla* and *Parmentiera cerifera* (Venkatasubban, 1944). The repeated occurrence of the value ten, for maximum association, would give additional support to the conclusion arrived at in a previous paper (Venkatasubban, 1944), i.e., that 10 is likely to be the primary basic number for the different species of the family Bignoniacae.

(d) *Anther tapetum*—

The anther tapetum was studied in *Dolichandrone Rheedii* and *Parmentiera edulis*. Mitotic divisions were seen in its cells and in fully developed condition, they were found to be multinucleate. As in the case of *Crescentia cujete* (Venkatasubban, 1944) fusion among the tapetal nuclei has been found to be a regular feature. Although the mature tapetal cells were apparently binucleate, these were really formed out of four nuclei which had fused in pairs. The tapetal cells were found to be much vacuolated and greatly elongated radially at the time of the division of the pollen mother cells.

Invariably, the tapetum was found to be of the intrusive type and both in *Dolichandrone Rheedii* and *Parmentiera edulis*, the microsporangial cavity was sometimes partitioned into two by the ingrowth of the tapetum. In *Dolichandrone Rheedii* (Text-fig. 34 and Photomicrograph, Plate VI, Fig. 1) the partitioning septum was found to be at one end of the anther while in *Parmentiera edulis* (Text-fig. 35) it was found to be right in the



Text-Fig 34 Part of an anther loculus of *Dolichandrone Rheedii* showing a secondarily formed loculus with several pollen grains $\times 740$ Same as photomicrograph, Pl VI Fig. 1

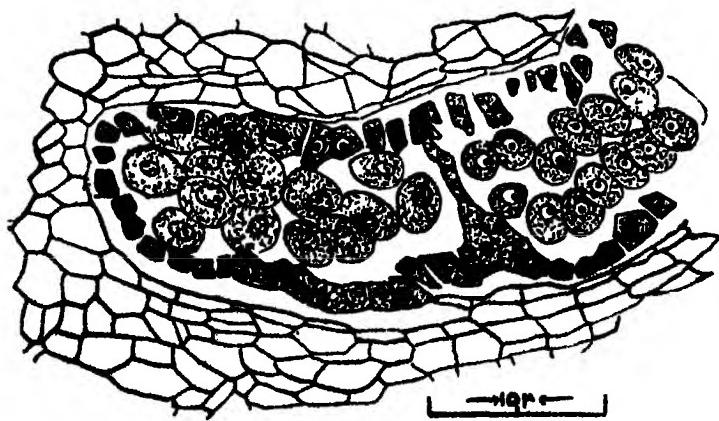
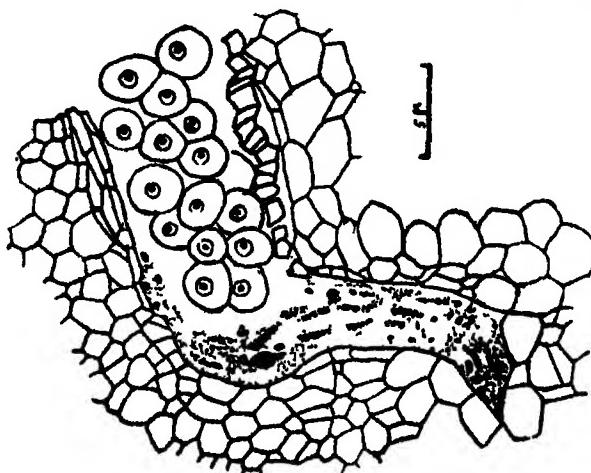


Fig 35. Part of an anther loculus of *Parmentiera edulis* showing the partitioning wall right in the middle and containing large number of normal P.M.Cs.

middle of the anther loculus. In both cases the pollen mother cells, contained in the two compartments were quite similar and numerous

Another interesting feature was observed in the anther loculus of *Parmentiera edulis*, where a sort of proliferation of the tapetum was noticed (Text-fig. 36 and Photomicrograph Plate VI, Fig. 2). The tapetum in this case was found to grow outside the loculus. It appears as though the



36

Text-Fig. 36 Part of an anther loculus of *Parmentiera edulis* showing proliferation of the tapetum; same as Photomicrograph Pl. VI, Fig. 2.

surrounding wall cells were gradually digested as a preliminary to the out-growth of the tapetal cells. The tapetum in this condition, at first sight, recalled the endosperm haustoria which form such a characteristic feature of the family. However the resemblance is only superficial as the two are developed in entirely different ways.

4. DISCUSSION

(a) Chromosome Numbers and Ploidy—

The somatic chromosomes of 7 out of the 8 species, belonging to the different genera, roughly agree in their morphology and all of them revealed a diploid number of $2n = 40$ chromosomes. *Spathodea nilotica* with $2n = 26$ alone being an exception. This identity of the chromosomes, in size as well as number, in a large number of the species belonging to different genera, may be interpreted as an indication of their monophyletic origin.

This study of the somatic chromosomes, besides bringing out the dominance of the number $2n = 40$ —a fact already recognized and emphasized (Venkatasubban, 1944), for the different members of this family,—also throws some light on the ploidy of the species. Thus in *Bignonia Unguis-cati*

4 prochromosomes were found attached to the nucleolus during early prophase and in *Tabebuia spectabilis*, a species belonging to an entirely different genus, 4 nucleoli were observed during early prophase in the root tip cells. These observations interpreted on the basis of the hypotheses of De Mol and Heitz (1928, 1931) seem to indicate that the two species in question are in the nature of tetraploids with a basic number of 10.

The tetraploid nature of the 40-chromosomed species is also supported by the occurrence of secondary association in *Dolichandrone Rheedii*. It has been pointed out already that the maximum association for this species is 10 [3 (1), 4 (2) and 3 (3)], from which it appears that in this case also the basic number is 10, i.e., the 40-chromosomed *Dolichandrone Rheedii* is a tetraploid derived from 10-chromosomed ancestors.

It should be of further interest to note that the maximum association and the basic number met with in *Dolichandrone Rheedii*, has also been found to be true for other members of this family like *Crescentia cujete*, *Tabebuia pentaphylla* and *Parmentiera cerifera*. On the basis of maximum association, as observed in *Dolichandrone Rheedii*, its gametic constitution may be put down as follows :—

AAA	BBB	CCC	DD	EE	FF	GG	H	I	J
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How such an association could be formed has been discussed at some length in the second paper of this series (Venkatasubban, 1944). Further it has been shown that in the evolution of the different species, structural alterations, such as segmental interchange without involving ring-formation have played a prominent part.

The next question is whether these 40-chromosomed species are allotetraploids or autotetraploids. The bulk of the cytological evidence is in favour of the former view. In meiotic studies of the various 40-chromosomed species, studied so far, no multivalent formation was seen at any time. Further, the second metaphase plates in most of the species showed an equal number of univalents, which could not be expected if there were any multivalent formation. The occurrence of secondary association, in a number of these species, would also be regarded as indication of the allotetraploid nature of these 40-chromosomed species.

(b) Anther tapetum—

It is generally believed, that the tapetal cells, both in the microsporangium and megasporangium, are concerned with the nutrition of the micro and megasporangium respectively. But from the point of view of structure, there is very little evidence to show that the tapetal cells are fitted either for

storage of food or for its transport to the developing spores. The nutritive rôle of the tapetal cells appears to have been assumed merely on some analogy with the endosperm cells.

The tapetal cells of a good many angiosperms are hyperchromatic. This is also true of the endosperm cells whose nuclei are usually triploid. Since the tapetal cells also contain more than one nucleus, it has been suggested by several authors, that they may also play a similar nutritive rôle.

It may be mentioned in this connection, that a multinucleate condition of the cells need not necessarily be associated with the process of nutrition. For example, the callus which develops around wounds or in galls, is mostly composed of polyploid cells (Darlington, 1937) and yet do not seem to play any nutritive rôle at all. Therefore, to conclude a nutritive function of the tapetal cells merely on the basis of their hyperchromaticity may not go with other observed facts. Again, it has been observed in a number of cases, that the tapetum may degenerate as early as the spireme stage. This is especially true of *Stenolobium stans* (Venkatasubban, 1944), although viable pollen grains are formed. Here, obviously, no nutritive function can be attributed to the tapetum which degenerates when it is most required.

Roener (1935), from his observations on the tapetal cells of *Hasta caerulea*, has given an explanation as to the manner in which stored food may be transferred from the tapetum to the pollen mother cells. He assumes that as the pollen mother cells develop, they exert a pressure on the tapetal cells so as to rupture them. The contents of the ruptured cells then flow into the anther loculus and provide nourishment to the developing pollen mother cells. According to Roener this nourishing fluid which flows into the anther loculus is yellow in colour. But his observations may not hold good for other plants, for, the yellow fluid, which is supposed to feed the pollen mother cells, was never observed in any one of the Bignonaceous species examined by me. Further, it is difficult to understand, how the developing pollen mother cells can bring about rupture of the tapetal cells. For, when the pollen mother cells enter into the heterotypic divisions, they generally become rounded up and this would release considerable space within the loculus, so that no perceptible pressure can be exerted on the surrounding cells of the tapetum.

There are several reports of the occurrence of an intrusive tapetum in angiosperms. This may well be interpreted as an attempt on the part of the tapetal cells to reach the pollen mother cells possibly with a view to nourish them. Or, it may be that the radial extension of some of the tapetal cells is purely due to limitations of space; for, they are closely packed and

as growth is pretty rapid in them, they simply grow in the direction of their long axes into the loculus of the anther which is not completely filled with the pollen mother cells.

Certain extreme cases of the formation of an intrusive tapetum were encountered in *Crescentia cujete*, *Dolichandrone Rheedii* and *Parmentiera edulis*. Here, the tapetum made its way right across the anther loculus, thereby, dividing it into two compartments. Similar instances were reported by Clausen (1926) in the F₁ generation of some *Viola* hybrids, who also noted that only a few pollen mother cells were included in the 'secondary loculus' and that these were as a rule larger and more regular than the rest. Wiger (1935) has also reported the occurrence of such partitions in *Chisocheton divergens* and that the one or two pollen mother cells included in a secondary loculus showed the formation of restitution nuclei. The significance of this formation is not clear. Probably this is due to hypernutrition. Choudhuri (1942) has also recorded this kind of partitioning of the anther loculus in *Limonium rariflora* and *L. Vulgare*. According to him the smaller compartment always contained only one pollen mother cell which was also found to be in a better condition than the rest.

This kind of tapetal behaviour (the partitioning of the anther loculus by the ingrowth of the tapetum right across it) may be regarded as an atavistic tendency which was once widely prevalent. For example, the microsporangia of *Isoetes* are traversed by a large number of septa which are similar to the tapetum. A similar tendency is seen among the angiosperms in some members of the Mimosoideæ (Rendle, 1930).

Another peculiarity that has been noted is the proliferation of the tapetum in a plasmoidal state (*Parmentiera edulis*). But, instead of finding its way into the anther loculus, the plasmodium had invaded the tissue surrounding the anther loculus, so that at first sight, it gave a striking resemblance to the endosperm haustoria which are so common in the family.

This raises the question, whether or not the tapetum can act as a haustorium in the same way as the endosperm. If the chief rôle of the tapetum is really the nutrition of the pollen mother cells, then the only way in which it can fulfil it efficiently is by assuming a haustorial function; but only further intensive studies on the tapetum of different plants can throw more light on this subject.

Further, the disappearance of the anther tapetum, during or after reduction division, may only be incidental. For, it may be assumed that the tapetal cells disappear or degenerate, either owing to hypernutrition or to their hyperchromaticity. The tapetum may therefore be regarded as a

tissue having only an ephemeral existence whose substance of disorganization is resorbed by the developing pollen mother cells. If this supposition be correct, the nutrition of the pollen mother cells may not have any direct relation to the tapetum.

From observations made on *Dolichandrone Rheediti*, and *Spathodea campanulata* (Raghavan and Venkatasubban, 1940), there are reasons to believe that the nutrition of the pollen mother cells can be brought about in other ways also. In the two species mentioned above, the pollen mother cells, even in the earlier stages of their development, were found packed with starch-like inclusions. These cell inclusions may go a long way in the matter of nutrition of the pollen mother cells without the aid of the tapetum. Further it frequently happens that not all the mother cells seen in an anther loculus mature into pollen grains. A good many of them perish and their remains, undoubtedly, serve as a source of food for the surviving pollen mother cells.

5 SUMMARY

The somatic chromosome numbers have been determined, for the first time, for the following species and were found to be as follows.—

<i>Bignonia Unguis-cati</i>	2n = 40
<i>Heterophragma adenophyllum</i>	2n = 40
<i>Markhamia platycalyx</i>	2n = 40
<i>Markhamia hildebrandtia</i>	2n = 40
<i>Stereospermum xylocarpum</i>	2n = 40
<i>Stereospermum suaveolens</i>	2n = 40
<i>Tabebuia spectabilis</i>	2n = 40
<i>Spathodea nilotica</i>	2n = 26

From the nucleolar behaviour, the number of prochromosomes attached to the nucleolus in some of the species during prophase and from secondary association studies, it is concluded that the 40-chromosomed species are in the nature of allotetraploids with a basic number of 10.

Secondary association was observed in *Dolichandrone Rheediti* and from the maximum association, the number 10 was derived as representing the basic number. The meiotic chromosome counts were made in the following species:

<i>Dolichandrone Rheediti</i>	.. n = 20
<i>Parmentiera edulis</i>	.. n = 20
<i>Tecoma serratifolia</i>	.. n = 19

The tapetum shows a considerable variation in its behaviour in the different members of the family Bignoniacae. In *Stenolobium stans*, it

disintegrates even before the pollen mother cells enter upon the meiotic divisions. In *Phyllarthron comorensis* also it begins to disappear at an early stage and no trace of it seen after the tetrad stage. In others like *Dolichandrone Rheedei* and *Parmentiera edulis*, the tapetum is of the intrusive type and its cells may protrude inwards to such an extent as to partition the anther loculus. A plasmodial type of tapetum is met with in *Parmentiera edulis*.

6 ACKNOWLEDGEMENTS

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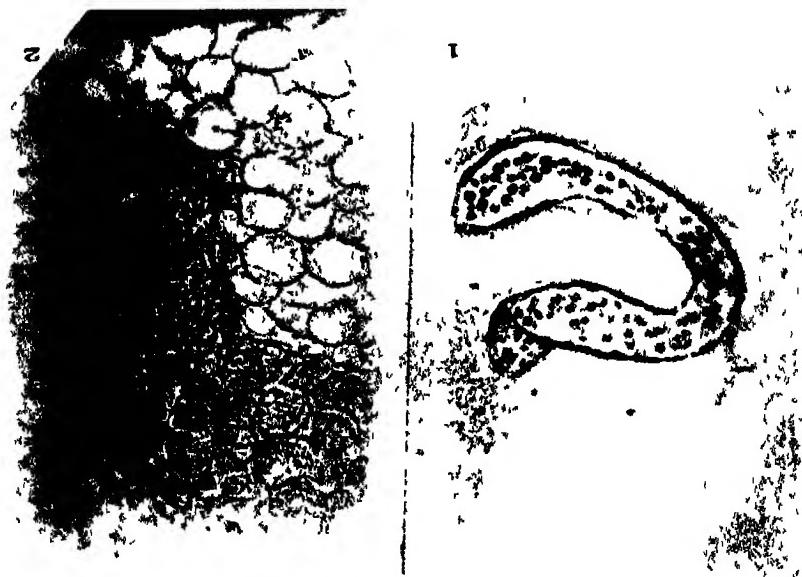
8 APPENDIX

List of Chromosome Numbers in Bignoniacae

Genus and Species	Chromosome numbers		Reference
	n	2n	
Tribe Bignonieae—			
<i>Bignonia Chereve</i> Lindl		40	Venkatasubban, 1944
<i>B. magnifica</i> Bull	20	40	do
<i>B. Chamberlainii</i> Sims.		40	do.
<i>B. megapotamica</i> Spreng	20	40	do
<i>B. diversifolia</i>		40	do
<i>B. purpurea</i> Hook		40	do
<i>B. gracilis</i>		80	do.
<i>B. Tweediana</i> Lindl.		80	do
<i>B. venusta</i>	ca25?		Duggar, B M., 1899
<i>B. Unguis-castri</i> Linn.		40	Venkatasubban, 1944
<i>Adenanthera polystachya</i>		40	do.

Genus and Species	Chromosome numbers		Reference Venkatasubban, 1944
	n	2n	
<i>Amphilophium Mustilii</i> H.B.S.K.		44	do.
<i>Tonacium diflorum</i> DC.		40	do
<i>Oroxylum indicum</i> Vent.		30	do
<i>Millingaria hortensis</i> Linn.	15	30	do
Tribe <i>Tecomeae</i> —			
<i>Tecoma stans</i> Juss.	20	40	do
<i>T. radicans</i> Juss.		40	do
<i>T. serratifolia</i> Don.	19		do
<i>T. chrysanthra</i> DC.		38	do
<i>T. grandiflora</i> Del		38	do
<i>T. rosea</i> Bertol. Pl. Guatim		38	do
<i>T. Smithii</i> W. Watts	18	36	do
<i>T. capensis</i> Lindl	17	34	do
<i>T. Tagalibana</i>	20		De Vilmorin and Simonet, 1927
<i>Dolichandra Rhodellii</i> Seem	20	40	Venkatasubban, 1944
<i>D. stipulata</i> Benth		40	do
<i>D. platycalyx</i> Baker		40	do
<i>Tebetida</i> sp. (Sibpur)		40	do.
<i>T. guyanensis</i> Hemsl		40	do
<i>T. rosea</i> DC.		40	do
<i>T. pentaphylla</i> Hemsl	20		do
<i>T. speciosissima</i> Nichols		40	do.
<i>Campsis radicans</i> Seem		40	
<i>C. grandiflora</i>		36	
<i>Incarvillea grandiflora</i> Bur and French	18		Sugiura, 1936
<i>I. Delavayi</i> Bur. and French		18	do
<i>Spathodea campanulata</i> Beauv.	13		Raghavan and Venkata- subban, 1940
<i>S. nitens</i> Seem		26	Venkatasubban, 1944
<i>Markhamia platycalyx</i> Sprague		40	do
<i>M. hildebrandtii</i>		40	do
<i>Heterophragma adenophyllum</i> Seem		40	do
<i>Jacaranda mimosifolia</i> D. Don		36	do
<i>Stereospermum chelonoides</i> Sims	20	40	do
<i>S. suaveolens</i> DC		40	do
<i>S. xylocarpum</i> Wight		40	do
<i>Pajonella Rhodellii</i> DC		40	do
<i>Catalpa syringifolia</i> Sims	20		
Tribe <i>Crescentieae</i> —			
<i>Parmentiera carifera</i> Seem	20	40	Venkatasubban, 1944
<i>Parmentiera edulis</i> DC	20	40	do.
<i>Crescentia cujete</i> Linn	20	40	do
<i>Kigelia pinnata</i> DC.	20	40	do
<i>Phyllarthron comorense</i> DC		40	do

Fig. 1 Photomicrograph of part of an inflorescence of *Brahmi amara* showing the formation of secondary locules containing several pollen grains in the early and
Fig. 2 Photomicrograph of part of the inflorescence of *Pannierella edulis* showing the proliferation



THE NATURAL OCCURRENCE OF ERGOT IN SOUTH INDIA

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THE occurrence of the genus *Claviceps* in South India has been recorded from time to time by various workers. McRae (1917) described *Sphacelia sorghi* on sorghum in the Madras Province. Ajrekar (1926) has recorded the same fungus on sorghum in the Bombay Province where he noted immature sclerotia invaded by *Cerebella*. Besides, he has observed *Sphacelia* on **Andropogon caricosus* var *molliccans*,¹ *A. annulatus*,² *Pennisetum alopecuroides*,³ and *Ischaemum pilosum* in the same province. Ergot sclerotia were observed on *P. alopecuroides*. Ramakrishnan (1937) has recorded *Sphacelia* on *Panicum ramosum* from Coimbatore. A *Claviceps* on sugarcane has been observed by Thirumalachar (1943) in Mysore.

Experiments on the production of rye ergot were carried out at the Agricultural Research Station, Nanjanad, Nilgiris, in 1941-42. These were successful and before venturing on an expanded scheme of ergot production, a survey was conducted on the Nilgiri plateau, portions of Wynad (Malabar District), Anamalais (Coimbatore District) and Kodaikanal (Madura District) to note the occurrence of indigenous species of *Claviceps*. The survey was fruitful and several grasses have now been recorded as being infected by the sphacelium and in some cases the sclerotial stages of *Claviceps*. These fungi have been recorded for the first time on these hosts excepting those on *Pennisetum Hohenackeri*, *Oplismenus compositus* and *Brachypodium sylvaticum*. The distribution is fairly widespread over the Nilgiris and Kodaikanal the same host showing infection by the same fungus in the two areas. Below are recorded short descriptions of the fungi and symptoms of the infection on the different host plants arranged according to hosts.

* The names of these grasses have been changed into:—

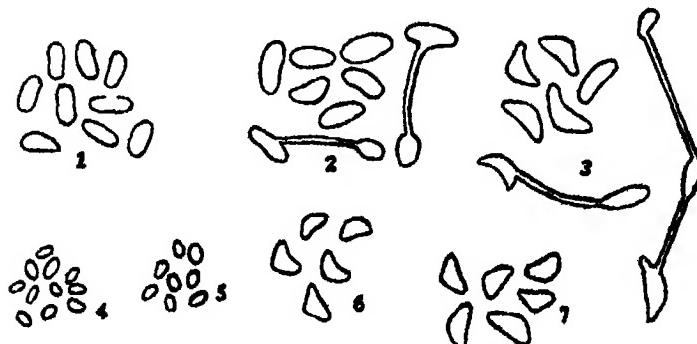
¹ *Dichanthium nodosum*

² *D. annulatum*

³ *Pennisetum Hohenackeri*

(1) *Cynodon dactylon*.—Occurrence: Coimbatore, Gudalur, Wynnaad (taluk of Malabar) and Naduvattam (Nilgiris) in the months of November to February. Sclerotia elongated, slightly curved $3\text{--}4\cdot5\text{ mm} \times 1\text{--}1\cdot5\text{ mm.}$, cream coloured when young but later becoming deep dark neutral grey externally and white internally (observed only at Coimbatore. Plate VII, Figs 5 and 6). Conidia oblong, elliptical or kidney-shaped, hyaline and measure $16\cdot2 \times 5\cdot7\text{ }\mu$ ($10 - 23 \times 3\cdot8 - 7\cdot5$). Conidia germinate readily in water with the production of secondary spores at the ends of germ tubes. Germination is very high between 60° and 70° F but very poor at 40° or 86° F .

(2) *Pennisetum Hohenackeri*.—Occurrence. Coimbatore and a widespread area in Wynnaad. Sclerotia small, cylindrical, black $3\cdot5 - 5 \times 0\cdot8 - 1\cdot8\text{ mm.}$ Conidia formed in light brown drops of viscous honey-dew which later dry into black crusty masses, curved, hyaline, with tapering ends and a prominent vacuole, $20\cdot4 \times 5\cdot8\text{ }\mu$ ($16 - 26 \times 3\cdot5 - 7\cdot5\text{ }\mu$); germinate readily producing secondary and tertiary conidia, germ tubes usually originate from the sides (Text-Fig 3). Ajrekar has recorded the occurrence of sphacelial and sclerotial stages on this grass from Bombay but no description is given of either of the stages. Conidia of similar type have been recorded on *Brachiaria ramosa* (*Panicum ramosum*) by Ramakrishnan (1937).



Text-Figs 1-7 Conidia from (1) *Themeda triandra* $\times 400$ (2) *Cynodon dactylon* (2 spores germinating and producing secondary conidia. $\times 450$). (3) *Pennisetum Hohenackeri* (also showing germination and formation of secondary and tertiary spores. $\times 450$). (4) *Oplismenus compositus*. $\times 400$ (5) *Amphilophis Fouquetii* $\times 400$. (6) *Crypsagapon zeylanicus*. $\times 400$. (7) *Heteropogon contortus* $\times 400$

(3) *Themeda triandra*.—Occurrence: Over most of the upper slopes of the Nilgiri plateau and in Wynnaad from July to February. Conidia, hyaline, oblong, often with a slight constriction in the middle, with granular contents the granules collecting into groups at either ends, $18\cdot0 \times 5\cdot4\text{ }\mu$.

($10\cdot5-17\cdot5 \times 3\cdot7-5\cdot5 \mu$), honey dew in the form of white semi-solid substance protruding out of the spikelet. No sclerotial stage observed.

(4) *Themeda quadrivalvis*—Occurrence. Kodaikanal and Nilgiri plateau from November to January Sclerotia elongated, cylindrical, attaining a length of 8 mm., lower portion black with a violet tinge externally, white inside, often tipped with the remnants of the white conidial mass (Plate VII, Fig 1); conidia oval or round, hyaline with granular contents $4\cdot0 \times 3\cdot3 \mu$ ($2\cdot3-9 \times 2\cdot2-6$), forming a whitish mass projecting out of the spikelet. Bits of sclerotia externally sterilised and transferred to tubes containing agar media produce a white mycelial growth. The growth is slow, thick and slightly folded.

(5) *Ischaemum aristatum*.—Occurrence Enjoys a wide distribution over most of the Nilgiri plateau, Wynnaad and Anamalais during the months of July-January. Conidia oblong or elliptical, rarely oval, hyaline, $12\cdot8 \times 5\cdot2 \mu$ ($7\cdot5-18 \times 3-6$); honey dew in the form of almost colourless drops at first, but later developing into a white deposit (Plate VII, Fig 9). Ajrekar has noted a Sphacelia with curved spores on *Ischaemum pilosum* from Bombay.

(6) *Amphilophis Foulkesii*—Occurrence Kodaikanal and several places on the Nilgiri plateau from October to February. Sclerotia cylindrical slightly curved, tapering towards the apex, grey in colour with a number of longitudinal grooves, $5 \times 1\cdot5$ mm., internally white (Plate VII, Fig 4). Conidia oval to oblong, hyaline $4\cdot6 \times 2\cdot8 \mu$ ($1\cdot5-9 \times 1\cdot5-4\cdot5$); honey dew protruding as a white mass out of the spikelet (Text-Fig 5). The fungus can be readily brought into culture from bits of sclerotia. A white growth with plenty of aerial mycelium develops and large numbers of conidia are formed.

(7) *Amphilophis insculpta*—Occurrence Coonoor (Nilgiris District) in January–February. Sclerotia similar to those formed on *A. Foulkesii* upto 6×1 mm., conidia oval, elliptic or oblong and measure $3-7 \times 1\cdot5-3 \mu$.

(8) *Chrysopogon zeylanicus*—Occurrence All over the open places on the Nilgiri plateau and Kodaikanal, heavily infected from November to January. Sclerotia very prominent, laterally compressed, slightly falcate a mixture of grey and black outside, white inside, longitudinal grooves and fissures present, $9\cdot5 \times 1\cdot6$ mm. ($6\cdot16 \times 1\cdot2$ mm.) (Plate VII, Fig. 10). Conidia hyaline, almost triangular, with rounded corners $11\cdot1 \times 6\cdot4 \mu$ ($6\cdot8-15\cdot8 \times 3\cdot7-9$) (Text-Fig. 6). The fungus can be readily brought into culture from bits of sclerotia. The growth is thick and white, slow, folded

and spores are found. These spores are triangular or sometimes oblong. Ajrekar has observed a sphacelia with triangular spores on *Andropogon caricosus* var *molliscomus*. In the sclerotium obtained from *Chrysopogon zeylanicus* traces of the alkaloid (ergotoxine) have been noticed by Mukerji and De (1944).

(9) *Heteropogon contortus* — Occurrence In several places at the higher elevations on the Nilgiris, Wynad, Kodaikanal and Coimbatore. Sclerotia long and slender, greyish black, with the whitish remains of the conidial stage at the apex, white inside, upto $10\text{ mm} \times 1\text{-}2\text{ mm}$, not easily separating from the spikelet, conidia (Text-Fig. 7) mostly triangular, $14\text{ }6 \times 6\text{ }2\mu$ ($9\text{ }30 \times 3\text{-}9$), hyaline, forming a white mass just protruding out of the spikelet, often drying into flakes over the glumes. The fungus can be brought into culture from bits of the sclerotium. A white thick folded slow growth results, resembling that of the fungus from *Chrysopogon*.

(10) *Cymbopogon polyneuros* — A sphacelial stage alone noticed on this grass in many localities on the Nilgiris, honey dew at first as colourless or light coloured drops of fluid coming out of the spikelet, later forming a white mass between the glumes, conidia hyaline, almost triangular with rounded corners, measuring $10\text{ }4 \times 4\text{ }9\mu$ ($7\text{ }5 \times 3\text{ }6$) occurs in the months of December-January.

(11) *Cymbopogon flexuosus* — Sphacelial stage alone observed on this grass at Coonoor, and Ketti in January-February. Honey dew whitish, conidia hyaline, oblong $8\text{ }7 \times 4\text{ }4\mu$ ($6\text{ }12 \times 3\text{ }6$), sclerotia not observed.

(12) *Ophiopogon compositus* — On the Nilgiri plateau and the Pulneys (Madura District) between November and March. Sclerotia blackish grey with a green wash at the base where enclosed by the glumes, elongated, $4\text{-}10 \times 1\text{-}1\text{.5 mm}$ straight or curved, with longitudinal grooves on the surface (Plate VII, Fig. 7). conidia forming white to greenish-white mass, projecting out between the glumes, conidia almost hyaline, oval to elliptic, $4\text{ }5 \times 2\text{ }2\mu$ ($3\text{-}6\text{ }8 \times 1\text{ }5\text{-}3$) (Text-Fig. 4). Padwick and Azmatullah (1943) have described a new species of *Claviceps*, *C. viridis*, occurring on this grass at Simla. The fungus observed on the Nilgiris and Pulneys appears to be the same as judged from the descriptions of the conidia and sclerotia. Mukerji and De (1944) did not find even a trace of ergotoxine in the sclerotia collected from South India.

(13) *Digitaria chinensis* — Over a wide area at Kodaikanal in the months of November-December. Sclerotia $5\text{-}1 \times 5\text{ mm}$, spindle-shaped, dark grey with a violet tinge, projecting from between the lemma and palea.

Conidial mass whitish, translucent conidia varying in shape, oblong or curved, hyaline $16.4 \times 4.1 \mu$ ($9-24 \times 3.6$)

(14) *Urochloa reptans*—Occurrence in Palghat and Coimbatore in November Sclerotia small, 2×1 mm, cylindrical, dark brown in colour, conidial mass white rounded and projecting clearly out of the spikelet Spores hyaline, curved with one or more oil globules $17.1 \times 4.8 \mu$ ($15-24 \times 3.6$) A sphacelial stage has been recorded on this grass by Rhind (1928) from Burma *Cerebella* was very common on the infected spikelets

(15) *Agrostis pilosula*.—Common on the downs and roadsides on the Nilgiris (Ootacamund) in December-January The sclerotia are minute and enclosed inside the spikelets, $1-1.5$ mm \times $3-5$ mm, cylindrical or oblong, violet black, with longitudinal fissures (Plate VII, Fig 2) Conidial mass whitish, spores elliptic to oval, hyaline, $7.2 \times 3.9 \mu$ ($3-12 \times 1.5-6$) The infected spikelets can be detected only by close examination on account of the small size

(16) *Apluda aristata*—Occurs in Wynnaad in January sclerotia slightly curved, closely clasped by the glumes at the base, dark grey with longitudinal grooves on the surface, $2.5-4 \times 1.5-2$ mm, conidia are hyaline, curved or spindle-shaped, $10.9 \times 4.9 \mu$ ($7.5-15 \times 3-7.5$)

(17) *Andropogon lividus*—Sphacelial stage alone is present on this grass on the Nilgiri plateau, conidia, hyaline, oblong, $10.3 \times 5.8 \mu$ ($7.5-15 \times 3-6$)

(18) *Brachypodium sylvaticum*—Occurs in the higher regions of the Nilgiri plateau in October-December; sclerotia black, cylindrical, slightly bent, upto 8 mm, easily shed (Plate VII, Fig 8). conidia hyaline, oblong to elliptical, $7.4 \times 3.6 \mu$ ($3-15 \times 1.5-6$) The fungus can be brought into culture easily from bits of sclerotia A white dense growth develops on agar media Later it becomes cream coloured Large numbers of spores are formed The fungus was successfully inoculated on Black winter rye Padwick and Azmatullah (1943) have described *Claviceps purpurea* on this grass from Simla The fungus observed on the Nilgiris is possibly *C. purpurea*

It is interesting to note that two species of *Claviceps* observed in the neighbourhood of Simla are present on the Nilgiris and one on the Pulneys also though these places are thousands of miles apart Till very lately it was believed that ergot is not present in India. It is possible that the absence of a record of these for all these years may be only due to the want of a

critical survey. These fungi may enjoy wider distribution than what is known at present.

Another interesting feature that was observed both during the experiments on ergot production and in the survey was the constant association of two saprophytic fungi, viz., *Cerebella* producing an olive black growth on the infected spikelets and a *Fusarium* giving rise to a pink-red development on the sclerotia and sometimes on the entire spikelet. The former is present throughout the year but the latter makes its appearance only during the moist monsoon months. As a matter of fact these two fungi help a great deal in locating the infected earheads of grasses and can be considered as indicators of *Claviceps*, though they occasionally form a handicap in the production of ergot of rye by affecting the sclerotia. In nature these must have contributed much to keep down the spread of *Claviceps* on the Nilgiris. It has been observed by Langdon (1942) that *Cerebella* does not develop on the inflorescences of grasses unless there was previous infection by the honey dew stage of *Claviceps* and that it serves as an indicator and is responsible for keeping down ergot formation "The history of ergot can be traced through *Cerebella*"

Subramaniam (1921) has recorded the occurrence of *Cerebella* on different grasses in India. It must be presumed that all these grasses must have been previously infected by *Claviceps* and have to be considered as records of *Claviceps*. Judging by the localities from which the species of *Cerebella* have been recorded it can be definitely said that the genus *Claviceps*, enjoys a much wider distribution in India than has hitherto been believed. These observations lead us to expect *Claviceps* on more grasses, and over a wider area provided careful search is made in the proper season. The months of August to February (moist and cold weather months) are more favourable for *Claviceps* and search has to be conducted only during these months.

In this paper is recorded the occurrence of *Claviceps* on 18 hosts. On 12 of these grasses sclerotia develop while on the rest the sphacelial stage alone has so far been observed. A correct identification of the species of *Claviceps* can be attempted only after the germination of the sclerotia and the observation of the morphology of the stromata, asci and ascospores. The results of cross inoculation experiments may also give additional evidence. It has not been possible to germinate the sclerotia from different grasses during the period. Hence judging from the conidial characters a tentative arrangement into groups has been attempted as in Table I.

TABLE I

Nature of conidia	Range of conidial measurements in μ	Host plants
1 Conidia curved or fusoid, sclerotia formed— (a) Conidia smaller honey dew whitish	10.9-17.6 \times 4.6	<i>Cynodon dactylon</i> <i>Urochloa reptans</i> <i>Digitaria chinensis</i> <i>Apluda aristata</i>
(b) Conidia bigger, honey dew dark coloured	20.4 \times 8.8	<i>Pennisetum Hohenackeri</i>
2 Conidia mostly triangular, sclerotia usually formed	10.4-14.6 \times 4.9-6.6 6.8-15.8 \times 3.7-9	<i>Chrysopogon zeylanicus</i> <i>Heteropogon contortus</i> <i>Cymbopogon polyleuros</i>
3 Conidia small, oval or round, sclerotia developed	4.0-7.4 \times 2-3.9	<i>Brachypodium sylvaticum</i> <i>Agrostis pilosula</i> <i>Amphiphilus Poukensis</i> <i>Amphiphilus insculpta</i> <i>Themeda quadrivalvis</i>
4 Conidia bigger, oblong only sphacelial stage	8.7-13.0 \times 4.4-5.8	<i>Themeda triandra</i> <i>Ischaemum aristatum</i> <i>Andropogon lividus</i> <i>Cymbopogon flexuosus</i>

Of the above, the third group appears to be *C. purpurea*. At least three other species can be formed from the rest. Further studies on the germination of sclerotia and the extent of host range are in progress.

Our thanks are due to Sri C S Rajarathna Mudaliar, Assistant Agricultural Demonstrator in Mycology, for assistance in conducting the survey.

SUMMARY

A survey was conducted on the upper slopes of the Nilgiri plateau, the Pulneys (in the neighbourhood of Kodaikanal), Wynad (Malabar and Nilgiris) and portions of Anamalais to find out if any indigenous ergots are present. *Claviceps* was observed on 18 species of grasses. All of these except three are new records. On twelve of these both the sphacelial and sclerotial stages were observed. On the rest the sphacelial stage alone was present.

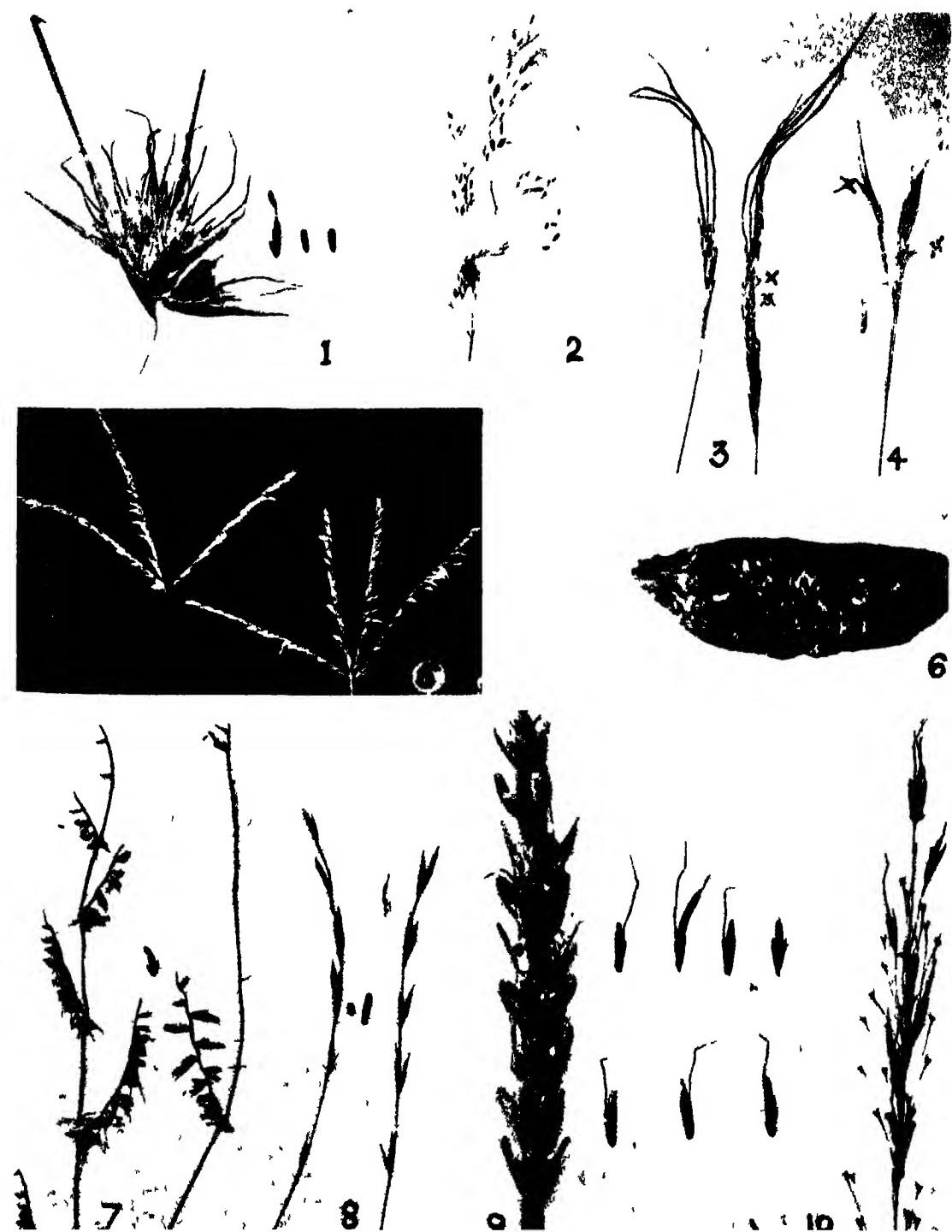
The fungi collected could be tentatively differentiated into five groups from the conidial characters. One of these appears to be *Claviceps purpurea*.

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EXPLANATION OF PLATE VII

- Fig. 1 A portion of the infected inflorescence of *Themeda quadrivalvis* and sclerotia
- Fig. 2 Infected inflorescence of *Agrostis pilosula*—sclerotia shown separate
- Fig. 3 *Heteropogon contortus* (sclerotia marked \times)
- Fig. 4 *Amphilophis Foulkesii* (sclerotia marked \times)
- Fig. 5 *Cynodon dactylon*—with several sclerotia
- Fig. 6 A single sclerotium from *Cynodon dactylon* $\times 100$
- Fig. 7 Inflorescences of *Oplismenus compositus* (several sclerotia are seen)
- Fig. 8 *Brachypodium sylvaticum* (sclerotium shown separately)
- Fig. 9 *Ischaemum aristatum* showing white conidial masses
- Fig. 10 *Chrysopogon zeylanicus* (infected spikelets with sclerotia shown separately)



STUDIES ON A VARIANT OF TRYPANOSOMA EVANSI IN A BUFFALO

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IN the course of examination of blood smears from a she-buffalo at the Veterinary Dispensary, Royapuram, Madras, Trypanosomes were met with and a study of their morphology disclosed differences from that of *Trypanosoma evansi*, the common causal agent of 'Surra'. The Organisms measured on an average 21 microns in length and 1-2 5 microns in width. The nucleus was not a compact body, but consisted of loosely lying bits of chromatin and situated a little anterior to the middle. The posterior end was blunt and rounded. The kinetoplast was at the extreme posterior end or very close to it. The axoneme started from the kinetoplast, crossed over to the convex border of the flagellate, traversed along the free border of the rather ill-developed undulating membrane and continued into a short free flagellum. A large number of granules dotted the cytoplasm, particularly anteriorly to the nucleus. The portion of the trypanosome anterior to the nucleus was narrowed down abruptly in width, so that the bulk of the protoplasm was behind the nucleus. This morphology of the trypanosome under study, combined with the absence of any other forms in the blood of the buffalo made the author feel he was dealing with a morphological variant of *T. evansi* or *T. vivax*. In order to ascertain the identity of the parasite experiments were undertaken to study the biological behaviour of the organism, as a difference in the morphology alone cannot be considered as a distinguishing character. Guineapigs, rabbits, white rats, mice, a sheep, a goat and a calf were used for this study.

Guineapigs—became infected rather slowly, the organisms being first seen in the blood on the 14th day after inoculation from the buffalo. The trypanosomes were very few even in wet films and even these disappeared in the course of two days from the peripheral blood. However, relapses occurred twice with an interval of 14 days on each occasion, when a few parasites were seen in the blood. The morphology of the very few organisms seen resembled that met with in the blood of the buffalo to a great

extent while forms identical to the *T. evansi* were in extremely few numbers. The duration of infection in guineapigs varied from 86 to 10 days the former duration when infection was effected from the blood of the buffalo and the latter when infection was effected by subpassages from rabbits and mice. This goes to prove the increase in virulence of the trypanosome when passaged through the laboratory animals

Rabbits—became infected within seven days after inoculation, the organisms being seen in fairly large numbers and fewer intervals of absence from the peripheral blood. The duration of the disease varied from 28 to 58 days. The morphology of the organisms seen in the blood of the rabbit was a mixture of a small percentage of forms resembling those seen in the buffalo blood while a large majority were of the type of *T. evansi*. It will be seen that rabbits appear more susceptible than guineapigs to the trypanosome under study and that the organism does change a little in its virulence in the rabbit with passage in laboratory animals.

White rats and mice.—The behaviour of the trypanosome in these two animals is exactly like *T. evansi*. There were a very few organisms resembling the ones found in the buffalo blood while the majority of the trypanosomes were similar to *T. evansi*.

Sheep.—A sheep was inoculated with blood from the rabbit showing a mixture of the two forms of trypanosomes. The flagellates appeared on the 7th day after inoculation and continued to be present for 5 days, at the end of which period (*i.e.*, 13 days after infection) the sheep died. The animal showed acute symptoms of surra and the post-mortem examination confirmed the disease. Here again, the blood contained both types of trypanosomes.

Goat.—A goat was inoculated with blood from the same rabbit as in the above experiment. Trypanosomes appeared in its blood on the 10th day and on the following days very few organisms were seen which consisted of the two types. A relapse, however, occurred after 5 days with a recurrence, later also. The animal lost condition gradually and the symptoms were suggestive of a subacute form of surra. The goat died on the 35th day after inoculation and the post-mortem lesions confirmed surra. The animal became much emaciated, unable to stand erect and later developed paresis of the hind legs before death.

Calf.—A calf was inoculated with the blood from the same source as the sheep and goat. Trypanosomes appeared on the 6th day of infection and never again, though the blood was infective to susceptible animals such as mice, killing them in a week to 10 days. The general condition of the calf

deteriorated and there was a relapse after six weeks, the trypanosomes being seen in very few numbers and consisting of both types. The calf had to be destroyed to avoid unnecessary suffering as it was in extremis in four months and seventeen days. A buffalo calf died in one month and twenty-two days after infection having shown symptoms of surra.

The result of the above study on the trypanosome reveals the following features among others:—

1. The morphology of the parasite as seen in the blood of this buffalo differs from that of *T. evansi* and suggests a similarity to *T. vivax*.
2. The infective inoculum from the original buffalo when passaged through laboratory small animals showed *T. evansi* (*Sensu stricto*) and a variant.

3. Guinea-pigs appear to be a little refractory in the beginning to the organisms taken directly from the buffalo; the virulence of the parasite becomes increased with passage through small animals.

4. Rabbits appear to be more susceptible than guinea-pigs, a feature which is different from that in *T. evansi* infection.

5. Sheep and goats, considered to be highly refractory to *T. evansi* infection, readily took up the infection and died out of it in the course of 15 and 47 days, respectively.

6. As regards the bovine too, the behaviour of this variant is not in accordance with infection by *T. evansi*, which usually induces a prolonged and refractory infection resulting in the ultimate clinical recovery of the animal. At least, artificially infected cattle do not always succumb to surra.

Microphotographs (Plate VIII, Figs 1 and 2) of the trypanosomes seen in the buffalo and of those usually met with in a case of surra in a bullock are reproduced for a comparative morphological study of the trypanosome.

DISCUSSION

Hoare (1938) in his paper on the diagnostic value of the kinetoplast, mentions that one to nine per cent of the forms in *T. evansi* possesses a terminally placed kinetoplast and 31–76 per cent in *T. vivax* have the kinetoplast placed at the posterior end. The trypanosome under study as found in the blood of the buffalo had cent per cent. of forms having a terminally placed kinetoplast. This is rather abnormal for the usual type of *T. evansi* met with in any animal. This variation in the morphology of the trypanosome combined with its abnormal behaviour to the laboratory small animals as well as to large animals such as sheep, goat, and calf makes the author feel that the

trypanosome under study is a morphological and biological variant of *Trypanosoma evansi*, if not, an entirely new species.

Bruce (1909) studying a trypanosome from Zanzibar, sent to him by Dr. Edington opined that it resembled *T. dimorphon* of Dutton and Todd and the trypanosome under study now agreed to a great extent with that one.

Stirling (1921) recorded a small trypanosome measuring 11 to 18 microns in length, from a bullock and was of the opinion that the organism found by him was of the *Dimorphon-congolense* group, which view was corroborated by the Director of Veterinary Education and Research of the Union of South Africa. It is unfortunate, however, that no biological studies were made by him on the trypanosome, in the face of the fact, that, that was the first record of any trypanosome of the *Dimorphon-congolense* group in India.

Rao (1931) in his observations on the trypanosomes of South India, mentioned, that the small trypanosome—*T. evansi*—occurring in the buffalo looks smaller than that found in the horse and observed that the measurements however tally with those (18-34 microns) given for *T. evansi*.

Rao and Mudaliar (1934) have made extensive studies on the trypanosomes affecting the domesticated animals in South India and concluded that the trypanosomes in all the cases resembled one another and were identical with *T. evansi*.

Thus it seems possible that there does exist a distinct morphological and biological variation in *T. evansi* which is so far considered to belong to the monomorphic group of trypanosomes along with *T. equum* and *T. equiperdum*. Probably, this trypanosome under study may approach the intermediary forms met with in the true polymorphic trypanosomes such as *T. brucei* and *T. congolense* of Africa, if not the short stumpy ones of the group. The absence of posterior nuclear forms in the guineapigs infected with this organism is, however, significant that it has no place among the true polymorphic trypanosomes.

It is proposed to name this trypanosome *Trypanosoma evansi* var. *rayi*, after Dr. H. N. Ray, Protozoologist, Imperial Veterinary Research Institute, Mukteswar-Kumaun.

SUMMARY

A small trypanosome was met with in the blood of a buffalo and its morphology differed widely from that of the usual type of *T. evansi*. A biological study of the organism was made on small laboratory and large animals, which also indicated that the trypanosome was different from *T. evansi*. It is considered that the organism studied is a variant of *T. evansi*.

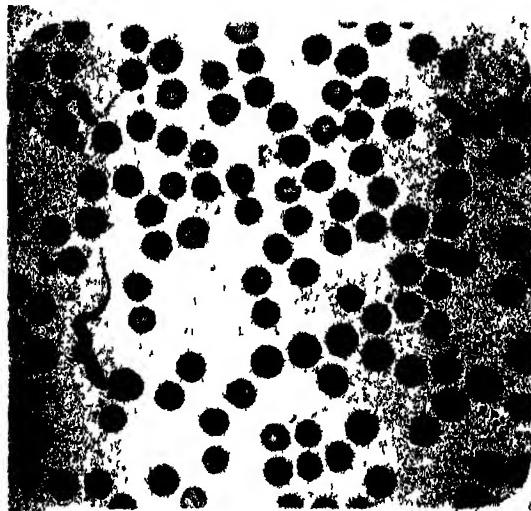


Fig. 1 Trypanosomes seen in the blood of the bullock under study

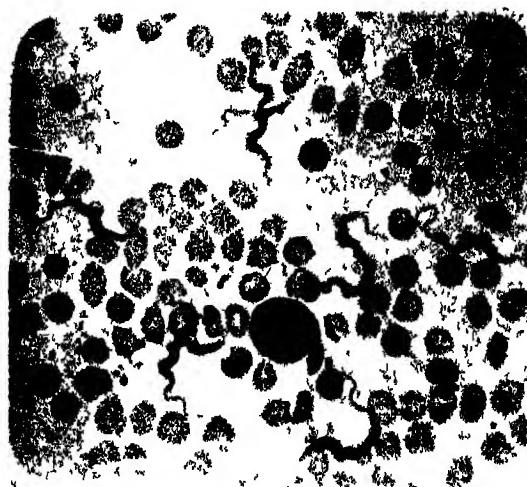


Fig. 2 *T. evansi* usually seen in the blood of a bullock with bovine surra

is not an entirely new one, and the author has named it *T. evansi* var. *rayi*, after Dr. H. N. Ray.

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SOME ASPECTS OF SPONTANEOUS SUBARACHNOID HÆMORRHAGE

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SPONTANEOUS subarachnoid hæmorrhage is the term used to distinguish it from traumatic and intracerebral hæmorrhages extending secondarily to the subarachnoid space. The term may not be very scientific and accurate, as in most of these cases there is rupture of intracranial aneurysms or diseased arteries. Very little attention was paid to it until the researches of Turnbull,²⁶ Fearnside⁹ and Symonds²⁷ were published. Though they have helped much to realise its cause, frequency and clinical recognition, our knowledge of intracranial aneurysm and subarachnoid hæmorrhage is still deficient. Like coronary thrombosis it may be considered as a "new" disease; for it is only during the past two decades that we have been able to recognise its incidence, clinical picture and consequences.

During the past four years I have had the opportunity to study and treat some cases of spontaneous subarachnoid hæmorrhage in the wards of the Sri Krishnarajendra Hospital, Mysore. It does not seem to be a very rare condition in our parts. The investigations are incomplete as I have been forced to confine my observations to the clinical and laboratory findings since post-mortem examinations could not be conducted.

ETIOLOGY AND PATHOLOGY

Though subarachnoid hæmorrhage may in rare cases result from various causes like trauma, purpura, whooping cough, acute infections, syphilis, arteriosclerosis and neoplasms like angioma, the most common cause is leakage or rupture of an intracranial aneurysm.

The numerous types of intracranial aneurysms are the traumatic, miliary, syphilitic, arteriosclerotic, mycotic and developmental or congenital. The miliary aneurysms are of no practical importance (Ellis¹ and Pick²⁸). Syphilis rarely causes intracranial aneurysms. Arteriosclerotic aneurysms may produce irregular dilatations of the intracranial arteries. Mycotic aneurysms may occur in bacterial endocarditis. "Developmental" or

"congenital" aneurysm is the most common type and it occurs at the angles formed by the bifurcation or branching of the arteries of the circle of Willis. It is sometimes termed as "bifurcation" or "berry" aneurysm. The term "berry" aneurysm is purely descriptive and noncommittal and has been aptly compared to the sprig of a mistletoe.

Berry aneurysms are usually single though they may be multiple. They vary between 3 to 8 mm in size. They may become adherent to the adjacent cerebral tissues, but it is more usual to find them free in the subarachnoid space. They seem to be more numerous around the anterior part of the circle of Willis, but in Martland's¹⁸ series they were distributed more or less symmetrically over the whole circle of Willis and its branches. In my cases the anterior part of the circle of Willis seems to be their site of election from the following facts: mental changes have been commonly seen, when hemiplegia occurred, the leg was more severely affected than the upper extremity; retinal haemorrhages were frequently found.

Microscopically the wall of the sac is entirely composed of fibrous tissue without any evidence of media or elastic tissue. Sometimes the wall of the sac gets calcified. A secondary mild periarteritis is probably the cause of the adherence of the sac to the adjacent cranial nerves. Minute extravasations of blood and haemosiderin may be found in the walls of the sac. Such extravasations suggest that these aneurysms may leak and cause subarachnoid haemorrhage by a gradual dissecting process. This is in accordance with the clinical history that subarachnoid haemorrhage is rarely caused by strenuous exercise.

The causes and modes of development of these aneurysms have not yet been completely elucidated. The circle of Willis is unique in the sense that it is supported in the interpeduncular fossa, where the hydrostatic support of the cerebro-spinal fluid must be an important factor in the regulation of the cerebral circulation. The impact of a double thrust on the communicating arteries with each cardiac systole may cause eddies and currents and thus subject them to greater strain. There may be some unknown mechanism to compensate for these resultant effects. The cerebral vessels belong to the medium sized, muscular group of arteries, and the elastica is mainly distributed in the intima. Eppinger⁸ suggested a congenital defect in the elastica. Turnbull¹⁹ and Forbus¹⁰ demonstrated local deficiencies in the media of the cerebral arteries at the point of junction of any two of the components the circle of Willis, or at, or very near the apices of the angles formed by the bifurcation of the cerebral arteries. These defects have been found by Forbus¹⁰ not only in adults and children, but also in the embryo.

He has found similar defects in the coronary and splenic arteries at points of bifurcation but they are less frequent. His hydrodynamic or manometric experiments in glass models proved that sudden increases in pressure were maximal at the points of bifurcation. The occurrence of medial defects at the bifurcation angles has been later confirmed by Strauss *et al.*²⁶ and Schmidt.²⁷ Forbus¹⁰ came to the conclusion, that though this congenital muscular defect is the primary basis of dilatation, degeneration of the internal elastic lamina caused by continued overstretching is the final stage in the formation of the sac.

Indirect evidence that these aneurysms are congenital is furnished by their occurrence in more than one member of the same family and by the occasional presence of other congenital abnormalities, such as coarctation of the aorta, cutaneous naevi and congenital polycystic disease of the kidneys.

There are certain difficulties in accepting that medial defect is the only cause in the formation of berry aneurysms. Berry aneurysms which are usually single, are very rare in infancy and childhood, uncommon in the teens, and more common as age advances. Medial defects which may occur in the intracranial, coronary and splenic arteries without the formation of aneurysms, cannot be the sole explanation of these aneurysms (Tuthill²⁸). Karmally and Manohar,¹⁶ though they believe that most of these aneurysms are congenital, are of opinion that medial defects by themselves do not explain the formation of aneurysms, and that the internal elastic lamina can maintain the integrity of the cerebral vessels in the presence of such medial defects.

Whether it is the structural or the degenerative change that determines the formation of aneurysms is not yet settled. Glynn¹³ has come to the conclusion that medial defects by themselves are of little consequence and that the concentration of the elastic tissue in the internal elastic lamina renders it more vulnerable to degenerative changes. All that can be said is that the structural bifurcation defects predispose to, and also determine the site of berry aneurysms. But there seems to be an unknown acquired factor other than hypertension and arteriosclerosis. Whether this factor is toxic or metabolic and how it acts on the internal elastic lamina is not known.

In the absence of post-mortem examinations, it is difficult to assert the cause of subarachnoid haemorrhage in my cases. Clinically syphilis, arteriosclerosis, subacute endocarditis have been excluded. Most probably, it was caused by berry aneurysms.

BRIEF CASE-HISTORIES ILLUSTRATING THE SYNDROMES OF SUBARACHNOID HÆMORRHAGE

Case 1 Hemiplegia—Recovery with residual defect—A Hindu male 52 years old, subject to "Epileptic fits" at long and irregular intervals from his young age, was admitted to the hospital on 16-10-1940, with a history that he had rather quickly become unconscious 12 hours before admission. On examination he was found to have hemiplegia on the right side

The patient improved remarkably in 24 hours and was able to speak a few words Pupils, fundi, heart, urine were all normal. B.P 162/86 Right sided paralysis with upgoing toe Provisional diagnosis—Thrombosis of the left middle cerebral artery.

Condition serious from the 19th He became comatose, pupils dilated and sluggish to light Left fundus large subhyaloid hæmorrhage C.S F uniformly blood stained, supernatant fluid-xanthochromic; W.R negative, Lange's test normal

After a critical week with signs of meningeal irritation like fever, cervical rigidity and a positive Kering's sign, he improved satisfactorily and became ambulatory after 6 weeks He was discharged home on 18-12-1940 with a normal C.S.F

The patient is alive and able to carry on his activities His right leg is a little weak. His moods are ever changing and he is irritable. He complains off and on, of headache The left eye is practically blind. The subhyaloid membrane had given way and resulted in vitreous hæmorrhage

Case 2. Hemiplegia with convulsions—Death from hyperpyrexia—A Hindu 63 year old male, well known for his intellectual activities, was admitted on 6-7-1943 with a history that he fell down unconscious ten hours previously He was addicted to alcohol On examination left-sided hemiplegia was discovered There were twitchings on the left side Left plantar reflex 'extensor' Pupils normal. Fundus arteriosclerotic changes, no hæmorrhages B.P 180/96 Urine no albumin or sugar Provisional diagnosis-left-sided hemiplegia

His condition became serious after 24 hours He became deeply comatose Pulse 124, B.P 194/108 Pupils dilated and sluggish to light. Right fundus congestion of the disc, small multiple hæmorrhages and a large blotchy hæmorrhage C.S.F uniformly blood stained, xanthochromic, microscopically, crenated erythrocytes

He seemed to improve after lumbar puncture and was able to answer a few questions; but on 10-7-1943, he developed uncontrollable convul-

sions, practically confined to the left side. A second lumbar puncture gave him no relief and he developed hyperpyrexia and expired on 11-7-1943.

This case, like case 1, is of interest in showing that fundal haemorrhages may not be evident during the first 24 hours. The cause of subarachnoid haemorrhage was probably degenerative arterial disease rather than berry aneurysm.

Case 3: Hemiplegia, meningeal irritation—satisfactory recovery—A Hindu landlord 50 years old was admitted to the hospital on 21-12-43 with a history that he could not use his right hand and leg. The onset was sudden with a feeling that something snapped in the head and he complained of headache. Twitchings were noticed on the right side for about two hours. He became dazed; but could speak indistinctly. When he was admitted he was unconscious. Right plantar reflex 'extensor'. Pupils unequal, moderately dilated. Left fundus small multiple haemorrhages; Right fundus normal. Pulse 90; Temperature 99.4°F; B.P. 160/92. C.S.F. blood stained and xanthochromic. The patient was drowsy for five days with occasional periods of consciousness. He had fever and resented disturbance. After a week he improved and was able to speak though rather indistinctly. He complained of headache and stiffness of the neck. He was wondering why he was brought to the hospital and wanted to go back home. During this stage he developed mild papilloedema in both eyes. By 20th January he was able to write. His right lower extremity, however, did not improve as quickly and as satisfactorily as his upper extremity, though he managed to walk by the end of the month. On 2-2-1944 the C.S.F. was normal except for slight lymphocytosis (24 cells per c.mm.) The papilloedema had not yet subsided completely. He left the hospital on 13-2-1944. His relatives say that he is more irritable after the attack.

These three cases show that hemiplegia is not an uncommon manifestation of subarachnoid haemorrhage. The aetiological factor is likely to be missed if repeated ophthalmoscopic examination and lumbar puncture are not done.

Case 4. Meningitic syndrome—Death—A Hindu male 40 years of age was admitted to the hospital on 23-2-1943. He had complained of severe headache on the previous day. He reeled and fell down unconscious while discharging his duties as a clerk in a law court from which place he was brought to the hospital. When he was in the hospital he was in a stupor from which he could be roused but his answers were not quite relevant. Bilateral upgoing toe. Pupils: small and active. Left fundus: disc margin hazy, multiple haemorrhages. Right fundus: normal. C.S.F. pressure increased, blood stained and xanthochromic.

During the next four days though he had meningeal irritation he improved. He had periods of consciousness on the 26th and 27th and could speak coherently. But he had a retrograde amnesia extending to the previous day.

Unfortunately his condition became suddenly worse on the 28th night after an injudicious attempt to get up from his bed. It is likely he was unable to realise its seriousness in spite of warning. On the 29th morning he was absolutely unconscious with dilated insensitive pupils. Left fundus: more haemorrhagic spots. Right fundus: a few haemorrhagic spots. He was taken home against medical advice on 2nd March. When it looked as though he might not survive his journey home, he lived for another 48 hours and was even able to speak.

This case is of interest in various aspects. Most probably it must have been due to subarachnoid haemorrhage from a leaking aneurysm at the anterior part of the circle of Willis. The mental symptoms might be due to the frontal lobe involvement. The occurrence of haemorrhage in the right eye after straining on the 28th night was probably due to a fresh bleeding. More interesting are the episodes of stupor alternating with periods of lucidity which seem to have no relation either to the blood pressure or C.S.F. pressure.

Case 5: Recurring coma—Recovery—A Hindu male mechanic aged 20 years was admitted unconscious to the hospital on the evening of 24-9-1943. The history was that while he was working on a lorry two hours before admission he suddenly complained of severe headache, felt giddy and fell down unconscious. A similar attack had occurred about two months before and he had regained consciousness in about a couple of hours. He had suffered from headache and pain in the neck for a few days, and resumed work within a week.

When he was seen on the 25th morning, he was comatose. The extremities were limp, the jerks were lost and the plantar reflex was 'extensor' on both sides. Corneal reflex abolished. Pupils irregular, moderately dilated and inactive to light. Right fundus: congestion of disc. Left fundus: congestion of disc, multiple haemorrhages and an old organised haemorrhage of 3 to 4 mm. C.S.F.—pressure increased, blood stained, xanthochromic.

His condition improved rapidly and in 24 hours he regained consciousness. He complained of headache, and was very irritable and resentful disturbance. By the 29th he felt so well that he wanted to go home. The C.S.F. drawn on the same day was more xanthochromic and contained less blood than on the 25th. Left fundus showed distinct swelling.

of the disc in addition to the haemorrhages. It was very difficult to convince him of the seriousness of his condition and he insisted on going away, saying that he felt perfectly well. However he was forced to stay in the hospital and he became quite reasonable after a few days and did not want to go home. Convalescence was uneventful On 12-10-43 the C.S.F. was clear and there were no erythrocytes and the cell count was 32 lymphocytes per c.mm A skiagram of the skull showed no abnormality The swelling of the disc had not yet subsided He was discharged home on 14-10-43. At present he is quite all right

This case is instructive in various aspects. It shows that xanthochromia can develop within 16 hours after the onset of subarachnoid haemorrhage and that it can disappear in about 18 days The old patch of haemorrhage seen in the left eye, probably resulted during the attack that had occurred two months previously The occurrence of late papilloedema is also interesting. The recklessness exhibited by him in demanding his discharge, only five days after the onset, is probably a manifestation of temporary abrogation of function of the frontal lobes This obstinate tendency disappeared in a few days. It is interesting to recall the recklessness displayed by men suffering from anoxia when working in rarefied atmospheres.

Cases 6, 7, 8 and 9 Coma—Death—These four cases were admitted from the out-patient department with a provisional diagnosis like cerebral malaria, diabetic coma, or epilepsy. All were males. Their ages were 24, 33, 36 and 42 years The history was that in all of the cases the onset of unconsciousness was almost sudden One of the patients had complained of headache, had vomited, and become unconscious in about ten minutes In none of them a history of unusual physical exertion was obtainable All these patients were literate and belonged to the lower middle class

On examination they were found to be deeply comatose. The blood pressure was not definitely raised Fundus showed small multiple retinal haemorrhages in all the cases In three cases it was bilateral Papilloedema occurred in three cases; it was bilateral in two and unilateral in one In one case there was a subhyaloid hemorrhage. The C.S.F. was uniformly blood stained in all of them and faintly xanthochromic in three There was retention of urine The catheterised specimen showed small quantities of albumin in two cases and heavy albuminuria in one case Two cases had sugar, one had acetone bodies. All the cases died within 24 hours after admission From the ophthalmoscopic and C S F findings there is no doubt that death was due to subarachnoid haemorrhage

Case 10 Preliminary cranial nerve paralysis—Coma—death.—A young man 27 years of age was referred to me for attacks of "migraine" on the

left side These attacks were occurring at irregular intervals for three months before he came under my observation They were strictly confined to the left frontal region He had intermittent diplopia for a week On examination, there was a slight prominence of the left eye, paresis of the third nerve and diminution of corneal reflex on the left side The left fundus was congested A provisional diagnosis of meningo-vascular syphilis was made, but the W.R was negative An alternative diagnosis of non-specific orbital cellulitis, causing the syndrome of the sphenoidal fissure was also considered He was prescribed a course of mercury and iodides He improved with this treatment and so did not seek further medical aid Three months later, after a strenuous day, he complained of severe headache, felt sick, vomited three times, took to bed and soon became unconscious He was deeply comatose, with wide and inactive pupils, and abolition of all the reflexes Fundi bilateral haemorrhages with intense congestion of the left retina He expired before a lumbar puncture could be done

This case is of interest as there were warning signs Though not confirmed by lumbar puncture, there is no doubt, from the history and the terminal attack with the retinal findings, that it was a case of subarachnoid haemorrhage.

Signs and symptoms—These can be considered under two headings. (1) before rupture, (2) immediately after rupture

(1) There may be no symptoms at all and berry aneurysms which in rare cases may grow to a large size and simulate slowly growing neoplasms, may be discovered during postmortem The symptoms depend upon the site and size They may cause symptoms by causing cranial nerve paralysis as in case 10. These may be evanescent and recurring They may also cause headaches, which may be migrainous The so-called ophthalmoplegic migraine has been attributed to congenital aneurysms Vascular disturbance may give rise to focal signs e.g., an aneurysm in the posterior part of the circle of Willis may impede the circulation in the posterior cerebral artery and give rise to homonymous hemianopia.

The diagnosis of these aneurysms before rupture is not easy. A skiagram of the skull may sometimes reveal them on account of the calcification in their walls (Albl's ring) There may be erosion of the bones. It is unusual to find any abnormality in a skiagram of the skull and there was none in any of my cases The only way of demonstrating them is by angiography after injecting thorotrust into the internal carotids and taking an instantaneous skiagram.

(2) *The mode of onset and the contributory causes for rupture of these aneurysms* are not well known Though they have a congenital basis,

they rarely rupture before adult life. That there is no relation to exertion in the large majority of cases is beyond dispute Magee,¹⁷ in his exhaustive survey of 150 cases, found that in 90% of them there was no history of physical effort and in 28% there was a special accent on rest He finds that exertion is not the exciting cause in cases of recurrence. But cases have been reported in which the haemorrhage seems to have occurred during unusual exertion—such as hitting a four at cricket, strenuous swimming, a tug of war, or running up the stairs The late Doctor Adie used to teach that “the commonest cause of ‘sunstroke’ in our country is subarachnoid bleeding” In none of my cases was there a history of severe physical exertion during which the bleeding might have occurred, but a history of rather unusual strain during the course of the day was obtained in two cases. The greater incidence in literate middle class males makes it probable that stresses and strains may have some indirect influence in causing subarachnoid haemorrhage The question, whether unusual exertion and mild trauma can cause it or not, is of great medico-legal importance when pensions and compensations are claimed In the large majority of cases the rupture of the aneurysmal sac is an insidious process of stretching and haemorrhagic dissection This fact is supported by finding haemorrhagic extravasations and haemosiderin in the walls of the sac In a few cases the tear may be precipitated by sudden fluctuations of blood pressure owing to violent exertion

Symptoms.—The manifestations of subarachnoid haemorrhage are protean Several syndromes may occur They are usually described as apoplectic, recurring coma, meningitic and migrainous

The most constant and usually the first symptom is headache The onset is sudden and may be associated with a feeling as though something snapped in the head It is usually general in distribution, but more often occipital than frontal It has often a tendency to radiate down the back of the neck Vomiting may occur soon after the headache and some patients may feel giddy and disoriented Convulsions may occur Loss of consciousness occurs soon after the headache in some severe cases and since a history of headache may be unobtainable it may be practically considered as the only initial symptom If the bleeding is not copious, consciousness may be dimmed or lost in a few hours after the onset of headache.

Physical signs.—The chief physical findings are those of meningeal irritation which may go on for several days The most common and constant physical sign is stiffness of the neck, not uncommonly associated with a positive Kernig’s sign. Though movement of the neck is painful, it is unusual to find any marked retraction of the head. The mental condition

of the patient in mild and moderate cases is one of retardation of memory, perception and attention. He is irritable and resents disturbance. In cases of frank bleeding, the patient may be comatose. Variability of the clinical findings is an interesting feature. At one time he may be conscious and carry on a lively conversation; at another time he may be restless and irritable or frankly unconscious. The deep and superficial reflexes may also vary from day to day. These fluctuations do not seem to have any definite relation to the C.S.F. pressure or the blood pressure. They may be due to a reactionary cerebral oedema. In very severe cases an acute compression is caused by the effused blood clogging the arachnoidal granulations, recalling Weed's experiments. Such cases are deeply comatose, and a fatal issue is probably not long delayed. Since the mental symptoms described above usually disappear after recovery they are the effects of subarachnoid bleeding and not of gross cerebral damage. The reflexes are usually sluggish. Unilateral or bilateral "extensor" plantar response may be found.

Moderate pyrexia is the rule. There is often a mild leucocytosis. The erythrocyte sedimentation rate was moderately increased in two cases.

Ocular manifestations—The pupils may be unequal and sluggish to light. The size and reactions of the pupils may vary from day to day. The corneal reflex may be diminished if the ophthalmic branch of the fifth nerve is involved, as in case 10. Exophthalmos may also occur before rupture as in case 10, and if it is present it may be of localising value. Proptosis sometimes develops rapidly, probably due to sudden venous congestion in the orbit. Photophobia is common and may make it very difficult for a proper ophthalmoscopic examination. There may be some changes in the visual fields. Permanent hemianopia may occur (Adie¹). Paralysis of the 3rd, 4th and 6th nerves may occur.

Ophthalmoscopic examination should be done in all cases. It is common to see multiple retinal haemorrhages which are usually small and flame-shaped and distributed near the disc; they may also be large and blotchy. Large subhyaloid haemorrhages are not rare, as in case 1. The subhyaloid membrane may rupture and result in vitreous haemorrhages. The retinal haemorrhages may be unilateral or bilateral. I find that they are more prominent in one eye than in the other and may be of localising value. In the hemiplegic cases (1, 2 and 3) the fundal appearances were prominent on the same side as the cerebral lesion. These changes may only develop a day or two after the onset as in cases 1 and 2. In acute comatose cases, the retinal haemorrhages tend to be bilateral. Out of the four fatal cases (6, 7, 8 and 9) it was bilateral in three.

Papilloedema, which is usually mild, should be looked for carefully. It may become apparent within a few hours. It seems to be more common in the acute comatose cases. Out of the four fatal cases (6, 7, 8 and 9) it was bilateral in two and unilateral in one. The occurrence of late papilloedema is difficult to explain. It may develop several days after the onset and may take weeks to subside (cases 3 and 5). It does not seem to be related to the blood pressure or the C.S.F. pressure or to correspond to the improvement of the patient. It may be related to the encystment of subarachnoid hemorrhage.

The pathogenesis of these retinal haemorrhages is uncertain. Anatomically it is not possible for the blood to pass from the optic sheaths to the retina through the lamina cribrosa. Riddoch and Goulden²³ suggested that the retinal haemorrhages were the result of an acute compression of the central vein of the retina by the blood that had passed into the optic sheaths. But haemorrhages in the retina may occur without any blood in the optic sheaths. Moreover, haemorrhages which have been found in the sclera, and even in the orbit cannot be due to the compression of the central vein. By taking serial sections, Ballantyne² has shown that the haemorrhages in all the layers of the retina and in front of it as well as in the vitreous are not in continuity with one another, but are discrete and independent. He believes that the haemorrhages are the result of a sudden increase in the intracranial pressure causing venous stasis in all the channels which drain the eye and the orbital contents. He also suggests that some manifestations like conjugate deviation of the eyes and oculomotor paresis, may be the result of small haemorrhages in the midbrain, produced by the sudden increase in the intracranial pressure.

The disturbance to venous return which occurs in subarachnoid haemorrhage and gives rise to papilloedema and haemorrhages in the fundus, is probably governed by the initial severity of the bleeding, the site of rupture and the presence or absence of arachnoidal adhesions. Fundal changes seem to be more common when aneurysms near the anterior part of circle of Willis rupture rather than when the aneurysms of the posterior part rupture. In cases 1 and 2, no fundal haemorrhages were observed on the first day, but only on the next day. Probably they were precipitated by fresh bleeding.

Fundal changes were observed in all my cases. This has not been the experience of other clinicians. It has been my practice to do a lumbar puncture in all the cases when retinal haemorrhages have been observed. It is probable that if lumbar puncture is done as a routine, even in the absence

of retinal hæmorhages, a few more cases of subarachnoid hæmorrhage may be detected.

The blood pressure—There was no definite elevation of blood pressure in any of the cases except in case 2. In this case the elevation was moderate, and possibly, the bleeding was from a degenerate artery.

Urinary findings—In comatose cases there may be retention and incontinence of urine. The urine may contain albumin, sugar and ketone bodies. Microscopically, erythrocytes, leucocytes and casts may be found.

The albuminuria and glycosuria which seem to be more common in comatose cases, may be quite massive and cases are mistaken for uræmic or diabetic coma. The mechanism of albuminuria and glycosuria is unknown. It is said that ketone bodies are present in such small quantities that they can be detected only by Rothera's test and not by Gerhardt's (Woolley³¹). In one of my comatose cases both the tests were answered. Though there may be moderate glycosuria there may be little or no elevation of blood sugar. This fact was confirmed in two cases.

The C S F Pressure—Immediately after hæmorrhage the pressure rises and if the bleeding is quite free death may ensue from acute compression. In moderate cases as soon as the bleeding stops, compensatory mechanisms come to play, and the intracranial pressure probably gets adjusted to an optimum. From the uniformity of readings it has been assumed that the lumbar pressure is representative of the changes in the intracranial pressure, though it is possible for the effused blood to interfere with the communication from the ventricles to the spinal theca. Manometric readings taken in two cases were found to be slightly elevated. In the long drawn-out stage of meningeal irritation the C S F pressure may not have any relation to the clinical picture.

Cellular and pigmentary changes—A detailed study of the pigmentary and cellular changes in the C S F was first made by Froin¹¹. The spinal fluid is evenly blood stained as revealed by collecting it in three test tubes. It does not clot on standing. On centrifuging, the supernatant fluid may be clear in the first few hours, but soon becomes xanthochromic owing to haemolysis. After a few days the xanthochromia may become more pronounced and in about three weeks it becomes clear and colourless. In case 5, xanthochromia was observed within 16 hours of the bleeding and it disappeared in 18 days. Microscopically it is usual to find crenated erythrocytes. After about two weeks an increase in the leucocyte count (mainly mononuclears) may be found. It may persist for weeks. In cases 3 and 5, the cell count was 24 and 32 lymphocytes per c.mm. after 6 weeks and 3 weeks respectively.

This may be due to meningeal irritation. The protein content is raised. Lange's test does not give any characteristic reaction. The W.R. is usually negative.

Diagnosis—Before rupture—In the majority of cases it is not possible to diagnose berry aneurysms before rupture. If the aneurysm grows to a large size, it may simulate a neoplasm, but it is a very slowly progressive lesion; the signs and symptoms of increased intracranial pressure are not prominent and the disabling focal symptoms are strictly localised. Cranial nerve palsies may occur. When a third nerve paralysis occurs in a young person berry aneurysms should be considered. Other causes of cranial nerve palsies like syphilis, and neoplasms should be excluded. The importance of plotting the visual fields has been stressed by Jefferson.¹⁵ A skiagram of the skull, particularly angiography, is of great value in demonstrating the aneurysms.

After rupture—The sudden onset of severe headache and prostration in a previously healthy adult with signs of meningeal irritation should arouse the suspicion of subarachnoid haemorrhage. If in such a case there are haemorrhages in the fundus, the diagnosis of subarachnoid haemorrhage is almost certain and can be confirmed if the C S F contains blood and exhibits xanthochromia.

The meningeal irritation and pyrexia may simulate meningitis. But the mode of onset, the fundal appearances and the C S F findings establish a correct diagnosis. In comatose cases, other causes of coma must be excluded. If sugar and ketone bodies are present in the urine, cases may be mistaken for diabetic coma. The history and mode of onset of diabetic coma are quite different. The intra-ocular tension in diabetic coma is low, and the patient is obviously dehydrated. Fundal examination and lumbar puncture will clinch the diagnosis. In cases of hemiplegia without any obvious cause like neurosyphilis, hypertension or mitral disease, a ruptured berry aneurysm should be thought of, and a diagnostic lumbar puncture resorted to.

The diagnosis of primary subarachnoid haemorrhage from intracerebral haemorrhage is difficult in some cases, particularly when it extends into the subarachnoid space. According to Merritt,¹⁶ the occurrence of convulsions or coma at the onset, and the development of focal signs like hemiplegia, are in favour of an intracerebral haemorrhage. My observations do not support this view. In case 2, convulsions were prominent and in case 3, there were twitchings. Coma may occur very rapidly in subarachnoid bleeding. Hemiplegia was a fairly prominent manifestation in cases 1, 2

and 3 The following points may be useful in differentiating the two conditions In subarachnoid hæmorrhage the patient is likely to be younger; there is no vascular hypertension and signs of meningeal irritation are more prominent A history of intense headache before the onset of coma is in favour of subarachnoid hæmorrhage If subarachnoid hæmorrhage is secondary to intracerebral hæmorrhage, the patient is deeply comatose and a fatal issue is not long delayed, whereas, in primary subarachnoid hæmorrhage the patient may be conscious though the C S F may contain large quantities of blood In subarachnoid hæmorrhage multiple retinal hæmorrhages are common and the arteries are healthy In primary intracerebral hæmorrhage the retinal vessels may be sclerotic and fresh hæmorrhages are unusual

Most cases of subarachnoid hæmorrhage are due to the rupture of a berry aneurysm In elderly subjects, it may rarely be due to the rupture of a degenerate artery In very rare cases particularly in children and young persons it may be due to angioma The venous angioma are likely to be associated with congenital abnormalities like nævi on the face In arterial angioma a bruit may be heard Angiomata may cause convulsions Radioscopy may reveal calcification

Clinical localisation of berry aneurysms may be possible from residual defects For example homonymous hemianopia locates the lesion near the posterior cerebral artery, hemiplegia affecting the lower extremity more severely than the upper extremity locates it in the region of the anterior cerebral artery

Prognosis—About 50% of the cases are said to recover In Magee's¹⁷ series of 150 cases, 56% died and in Taylor and Whitfield's²⁸ series of 81 cases 63% died In my small series of ten cases 70% died It is difficult to assess the prognosis in any individual case, since we cannot predict whether the bleeding continues or recurs Arterial disease and hypertension make the outlook worse The outlook is serious if the patient does not show any improvement within 48 to 72 hours Profound coma is usually if not always, an ominous sign In my experience the presence of bilateral hæmorrhages in the retina, particularly if associated with papillœdema is of serious import This is probably because bilateral hæmorrhages in the retina are prone to occur in cases of severe bleeding

There are very few conditions, if any at all, in which the patient may be so acutely ill, and yet remarkably recover without any residual signs. Disabling sequels result in some cases Damage to the cerebral tissues may give rise to focal signs If the frontal lobes are involved mental manifes-

tations may be prominent. If severe, institutional treatment may be necessary. Usually they are mild and may manifest themselves in irritability of temper, obstinacy and lack of concentration (case 1). Headaches may occur as a sequel (case 1). Cases have also been reported in which recurring headaches disappeared after the attack. In hemiplegic cases there may be residual weakness (case 3). If the intra-ocular haemorrhage has been violent, the eye may be lost (case 1). Cranial nerve palsies may also occur. Other focal symptoms depend upon the site of damage.

Recurrence—A firm and efficient walling off by adhesions at the site of rupture may decrease the liability to further attacks. Efficient adhesions are more liable to occur if the enforced rest in bed is long enough for proper healing. Case 5 had a second attack in two months. It is difficult to say when and how many of the patients will have a recurrence. In Magee's¹⁷ series there were 30% recurrences, and the major incidence of the second attack fell within a month of the initial attack. The mortality rate in a second haemorrhage was double that of the first. Other investigators believe that the mortality in subsequent ruptures is not greater than in the initial haemorrhage. Strauss and Tarachow²⁸ have suggested that in the absence of generalised disease, a history of presumptive or proved previous attacks tends to make the prognosis of the presenting attack more favourable. Barlow³ reports the case of a male who survived eleven attacks of subarachnoid haemorrhage from an angioma between the ages of 12 and 26.

Treatment—The ideal to be aimed at is the recognition and treatment of the condition in the patient's home as further damage may be caused during transit (Gale¹²). There should be very little loss of time particularly in cases where death is threatened by acute compression. Since facilities for proper treatment and nursing are rarely obtainable in the patient's home it is best they are treated in hospitals.

The cardinal feature in the treatment is absolute rest in bed. The head of the bed should be a little elevated to reduce the intracranial pressure. There should be no tight clothing round the neck and kinking of the neck should be avoided. The rationale of this procedure becomes clear when we consider the sudden response of the intracranial pressure to Queckenstedt's test. The bowels should be kept open and straining at stool avoided. Liquid paraffin, liquorice and small enemata on alternate days will usually be sufficient to achieve this purpose. Restlessness and delirium can be allayed by efficient nursing and by the use of sedatives, particularly, luminal and bromides. It is best to avoid morphia.

To arrest the haemorrhage it is customary to use haemostatics. They are of doubtful value. Some clinicians advise venesection if the blood pressure is very high.

Spinal drainage—All are agreed that a diagnostic lumbar puncture should be done. The question of repeated drainage is controversial. Collier⁶ and Merritt¹⁹ advocate repeated drainage whereas Hall,¹⁴ Bramwell⁸ and Richardson and Hyland²² do not advise it. Some clinicians who first advocated it have later become more conservative. It is impossible to say definitely when lumbar puncture is indicated. Attempts have been made to base the indications on records of blood pressure. I am doubtful if it is of much value. Repeated lumbar puncture is suggested when the C.S.F. pressure is very high. As a rule this is not practicable as it is often very difficult to do a lumbar puncture. To depend only on manometric readings ignoring the clinical condition is not advisable. After a diagnostic lumbar puncture the best thing to do is to watch the progress of the patient. As long as he is improving there is no necessity to repeat it. If he does not show any improvement in 48 to 72 hours a second lumbar puncture may be cautiously done. If life is threatened by acute compression lumbar puncture is indicated. A severe and persistent headache is an indication for lumbar puncture. While the ordinary analgesic drugs and even morphia may fail to relieve the distressing headaches, lumbar puncture often gives dramatic relief. Apart from these indications, it is unnecessary to resort to repeated spinal drainage as a routine. There is no definite clinical evidence that it lessens either the mortality or the tendency to recurrence. Nor is there any evidence that the disabling residual effects are less severe in cases which have been so treated. There is evidence to show that the effused blood can be adequately dealt with in the subarachnoid space and that disabling meningeal adhesions do not occur as sequelæ when the blood in the C.S.F. is not drained. One must bear in mind the possibility of the danger of recurrence of haemorrhage by an injudicious lumbar puncture which might suddenly disturb the pressure relationships and open up the bleeding point.

Intravenous hypertonic solutions of glucose or better sucrose may allay the delirium and distress of the patient particularly in the spurious episodes of worsening during the long drawn-out stage of meningeal irritation.

Duration of strict recumbency—The patient should not be allowed to get up until he is free from all symptoms like headache, stiffness of the neck and dizziness for at least 2 to 3 weeks. Case 4 is an instance in which an injudicious attempt to get up might have caused a recurrence of bleeding.

Some patients improve so quickly that they demand their discharge (cases 3 and 5). It is dangerous to yield to their request, for it is possible that recklessness may be a manifestation of the condition.

After treatment. —Patients should be advised to limit their activities. Constipation, sudden exertion and exposure to the hot sun should be avoided. Elevation in the blood pressure, if any should receive proper attention. Disabling sequelæ should be treated.

Surgical treatment like ligation of the internal carotids, is not possible in the majority of cases, but in selected cases it may be necessary (Jefferson¹⁸).

SUMMARY

- 1 Ten cases of subarachnoid haemorrhage are reported
- 2 The aetiology, pathology, symptoms, diagnosis and treatment are outlined
- 3 The characteristic C S F findings and the indications for lumbar puncture are discussed
- 4 The importance of ophthalmoscopic examination has been stressed

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ON TWO NEW HÆMOPROTEIDS OF INDIAN BIRDS

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INTRODUCTION

INDIAN birds harbour in their blood a rich and varied parasitological fauna, particularly the Hæmoproteids. We owe our earlier knowledge of this genus in Indian birds to Donovan (1904), Stephens and Christophers (1908), Acton and Knowels (1914), Adie (1915), Plummer (1912-16) and Scott (1925). Forty-one different species of birds were studied by these workers and twenty-one new species of Hæmoproteus were recorded.

Then followed the researches of de Mello (1916-17 to 1935-38) and the rich ornithological fauna of Portuguese India was carefully studied by him. Ninety-one different species and varieties of birds were studied and thirty new species of Hæmoproteus were registered and specific names were given.

Malkani (1936) recorded a new species *H. rileyi* from an ailing peacock and suggested that this parasite can cause an illness which might prove fatal. Maqsood (1943) also records a new species *H. handi* from a parakeet and he thinks that there was evidence to believe that the parasite could cause fatal protozoan disease.

The researches of the above-mentioned workers and the exact rôle played by these birds in the transmission of diseases to poultry tempted me to take up a preliminary investigation. Twenty-one birds including fifteen species* were shot during the month of December 1943, January, February and March 1944, in the area of the reserved forest and the Government Poultry Farm, Telinkhery, Nagpur. The present note records the presence of three Hæmoproteids including two new species in the following birds —

- 1 *Anthus r. rufulus*
- 2 *Merops orientalis*
- 3 *Xantholema haemacephala*

H. anchi in *Anthus r. rufulus* has already been recorded and a specific name has been given by de Mello (1935). No hæmoproteids have yet been recorded in the other two birds.

* Fifteen different species of birds which were studied at Nagpur — *Argya Malcolmi*, *Anthus r. rufulus*, *Acridotheres tristis*, *Centropus sinensis*, *Copsychus chus s. salurus*, *Hypolais rama rama*, *Merops orientalis*, *Oriolus oriolus*, *Passer domesticus*, *Phoenicurus ochruros*, *Rhipidura aureola*, *Streptopelia suratensis*, *Saxicola caprata*, *Tenenuchus pagodarum*, *Xantholema haemacephala indica*.

MATERIAL AND METHODS

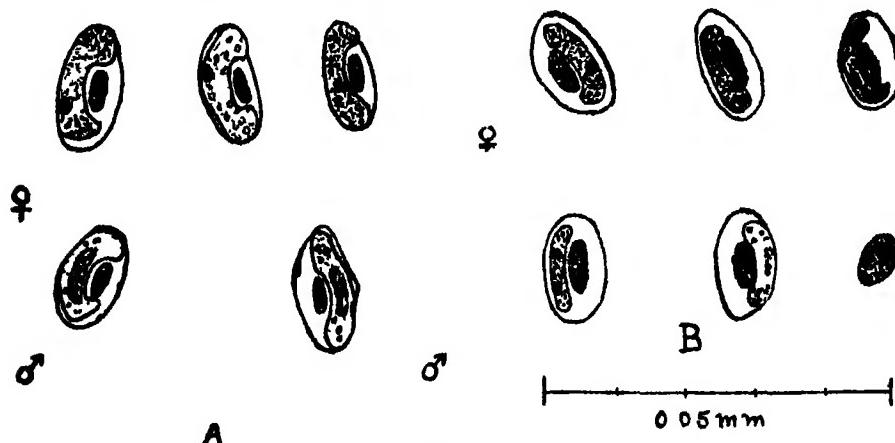
Immediately after the birds were shot blood films were made. Impression smears were also made from different organs namely, lungs, liver, spleen, etc. Smears were stained with Romanowsky (May Grunwald Giemsa or Leishman). Careful Camera lucida drawings were made.

OBSERVATIONS

1 *Hæmoproteus meropi*, n sp (Fig A)

Female gametocyte mostly bean-shaped, or halteridial, markedly vaculated protoplasm, staining deep blue by Romanowsky. Nucleus compact, pale rose, central or sub-central. Pigment brownish black in granules or rodlets of different size scattered all over the body often grouped in clusters. Size 10 to 16 microns.

Male gametocyte, very few, vermiform or halteridial homogeneous colourless or light blue cytoplasm. Nucleus relatively large somewhat



A *H. meropi*, n sp (of *Merops orientalis*)

B *H. xantholæmi* n sp (of *Xantholæma hemacephala*)

stranded, pale rose, pigment more of black in granules of different size scattered all over the body. Size 8 to 15 microns.

Host—*Merops orientalis* (green bee eater)

Locality—Area of Government Poultry Farm, Telikhery, Nagpur

2 *Hæmoproteus xantholæmi*, n sp (Fig. B)

Female gametocyte mostly oval or halteridial, protoplasm slightly alveolar, staining light blue by Romanowsky. Nucleus rosy, Central or sub-central. Pigment brown black of varying size independent or in clusters collected at poles. Size 7 to 13 microns.

Male gametocyte mostly oval, embracing the nucleus, staining light blue at poles by Romanowsky Nucleus rosy, stranded, practically filling the central portion and giving rosy tinge to the parasite. Pigment brown black of varying size usually collected at the poles. Size 7 to 12 microns

Host — *Xantholæma hæmacephala* (Indian crimson breasted barbet Syn. Copper smith)

Locality — Reserve forest, Telinkhery, Nagpur

DISCUSSIONS

Workers on this genus so far hold the view that on pure morphological grounds there are enough characters to individualise, as distinct species, the different Hæmoproteids found in various birds. At this stage it can safely be stated that every ornithological genus has its own species. Probably birds of the same genus harbour the same hæmoproteids or its varieties. The characteristic halteridium shape, the presence of pigment granules and essentially the absence of schizonts in blood films, confirm that these intracorporeal parasites belong to the genus *Hæmoproteus*. So the species which occur in the *Merops orientalis* and *Xantholema hæmacephala* may be held as two new species and the author has given them the specific name according to the ornithological genus to which the birds belonged, to avoid any confusion.

Hence the two new species

1. *Hæmoproteus meropi*, n sp in *Merops orientalis*
2. *Hæmoproteus xantholæmi*, n sp in *Xantholema hæmacephala*

SUMMARY

Twenty-one birds including fifteen of different species have been studied and Hæmoproteus infection has been registered in three birds, in two of which the parasite has been recorded for the first time

1. *Family* — *Hæmoproteidae*

Genus — *Hæmoproteus*

Species — *Hæmoproteus meropi*, n sp

Hosts — *Merops orientalis* (green bee eater)

Locality — Area of Government Poultry Farm, Telinkery, Nagpur, C.P., India

2. *Family* — *Hæmoproteidae*.

Genus — *Hæmoproteus*

Species — *Hæmoproteus xantholæmi*, n.sp

Hosts — *Xantholæma hæmacephala* (Indian crimson breasted barbet Syn Copper smith).

Locality — Reserve forest Telinkhery, Nagpur, C.P., India.

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TREMATODES FROM INDIAN MARINE FISHES

Part I. On Some New Monogenetic Trematodes of the Sub-orders
Monopisthocotylea Odhner, 1912 and *Polyopisthocotylea* Odhner, 1912

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I. INTRODUCTION

THIS paper is one of a series of papers on the trematodes of marine fishes of the Indian coast. Parts II and III of this series dealing with new Gasterostomes and Prosostomes respectively have already been published (Chauhan, 1943). This paper contains description of some ectoparasitic monogenetic trematodes and surprisingly enough is the first record of this group of worms obtained from India with the exception of a form obtained from the gill chambers of two species of fresh water fishes, *Puntius puckelli* and *P. ticto* by Dr V N Moorthy from Chitaldrug and described by Price (1938) under the name *Dactylogyrus moorthyi* Price, 1938. In view of the fact that our knowledge of the Indian forms of this group of trematodes is limited only to one species*, I have ventured to make the present paper more elaborate than is usual for such type of work.

II MATERIAL AND METHOD

In November-December 1939 and December-January 1940-41, I examined at Bombay about two hundred marine fishes for parasites, all possible locations, e.g., intestine, gills, skin, mouth cavity were examined. Parasites were fixed in Bouin's Fluid and preserved in 70% alcohol. Thick forms were slightly pressed between two slides and then immersed in the fixative. The specimens were stained by Delafield's Hæmatoxylin, Borax carmine and Hæmalum and were differentiated, cleared and mounted in the usual manner. Hæmalum gave best results though Borax carmine was a better stain to bring out very prominently skeletal elements which remained absolutely unstained and thus could be easily made out.

The incidence of infestation was very low and usually one or two specimens were found on a fish. Some of the forms were very small and were usually passed over, some were very delicate and broke away in separating them from host tissues. Some forms specially Gyrodætylords, did not take stain well and little could be made out of their anatomy. The present paper deals only with well fixed, well stained and mature forms.

* Stewart (*Rec Ind Mus*, 1914, 10, 195-205) published a description of *Polystomum kachugei* n.sp from the urinary bladder of *Kachuga lineata* from Allahabad, now regarded as *Polystomoides kachugei* (Stewart, 1914), Ozaki, 1935. Dayal (*Proc Nat Acad Sci India*, 1941, 11, 93-98) published an account of *Diplozoon indicum*, n.sp from the gills of a fresh water fish *Barbus sarana* from Lucknow. But no monogenetic trematodes have been recorded so far from marine fishes of India.

III. DESCRIPTIONS OF NEW FORMS

Class—Trematoda Rudolphi, 1808

Order—Monogenea (Van Beneden, 1858) Carus, 1863

A *Sub-Order*—Monopisthocotylea Odhner, 1912

Super-Family—Gyrodactyloidea Johnston and Tiegs, 1922

Family—Dactylogyridæ Bychowsky, 1933

(a) *Sub-Family*—Tetraonchinæ Monticelli, 1903

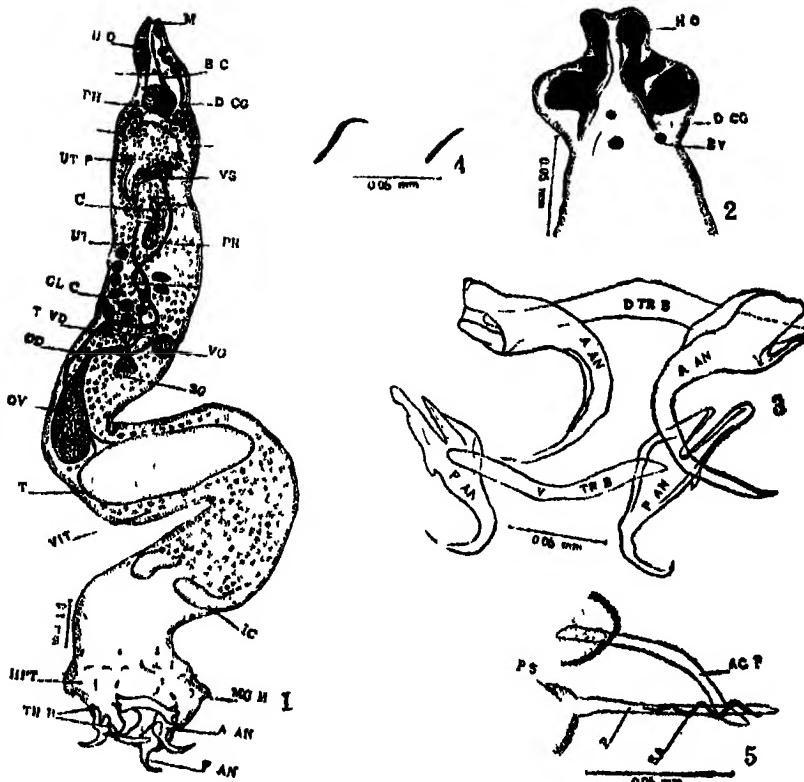
Genus—Ancyrocephalus Creplin, 1839

(1) *Ancyrocephalus alatus*, n.sp.

(Figs 1-5)

Numerous specimens of this parasite were found in the gill washings of marine fishes, *Muraenesox talabonoides*, *Arrius fulcarius*, *Mugil parsia* and *Harpodon neherius* both in November-December 1939 and December-January 1940-41. The incidence of infection was very heavy in *Muraenesox talabonoides* where every one of the specimens examined was found to be infected.

Body elongate (Fig. 1), anterior end tapering or wedge shaped, posterior broad and flat, lateral margins almost paralleled for the most part of the body length, very minute, delicate forms covered over with mucous, in the living condition, shape and size vary greatly—length being 0·71-2·43 mm and width 0·11-0·27 mm, greatest width in the region of testis, posteriorly the parasite ends in a well-defined adhesive disc, haptor (HPT) measuring 0·1-0·33 mm in width and bearing a dorsal (Fig. 3, A AN) and a ventral (P AN) pair of biramous laterally curved anchors; each pair of anchors with median dorsal and ventral transverse supporting bars (Fig. 3, D TRB, V TRB) to which the anchors are articulated laterally; average length of an anchor of the dorsal pair 0·17 mm and of its transverse bar 0·16 mm., an anchor of the ventral pair measures 0·12 mm in length and its transverse bar 0·12 mm: transverse bar of the dorsal pair usually curved anteriorly in the middle and that of the ventral pair posteriorly; marginal hooklets (Fig. 1, MG H) 12 in number, elongate, pointed or curved (Fig. 4), measuring 0·02-0·043 mm. in length. Cephalic glands (Fig. 1, CG) seven to nine, extending laterally on each side, or in a compact mass in a contracted animal, in the region of the pharynx, from which ducts enter the three head organs (Fig. 1, HO) on either side. In some cases the middle pair of head organs may be partially subdivided or the middle and the last pair may be even lobular (Fig. 2, HO). Two pairs of eyes (Fig. 1, EY) situated in the region of pharynx; anterior pair smaller in size and more approximated than the posterior. Pharynx (Fig. 1, PH) a small muscular, sub-spherical



FIGS 1-5 *Ancyrocephalus alatus*, n.sp.—Fig. 1 Entire view Fig. 2 Anterior end to show head organs and eyes Fig. 3 Dorsal and ventral pair of anchors with transverse bars Fig. 4 Two marginal hooklets Fig. 5 Penis with spiral ala, accessory piece, and a portion of the penis sac

A AN, Anterior anchor, *AC P*, Accessory piece of penis, *BC*, Buccal cavity, *C*, Cirrus, *CG*, Cephalic glands, *DCG*, Duct of cephalic glands, *D TR B*, Dorsal transverse bar, *EY*, Eye, *GL C*, Gland cells, *HO*, Head organs, *HPT*, Haptor, *IC*, Intestinal cæcum, *M*, Mouth, *MG H*, Marginal hooklet, *OD*, Oviduct, *OV*, Ovary, *PAN*, Posterior anchor, *PH*, Pharynx, *PR*, Prostato reservoir, *P*, Penis, *PS*, Penis sac, *SA*, Spiral ala, *SG*, Shell gland, *T*, Testis, *TR B*, Transverse bar, *TVD*, Transverse vitelline duct, *UT*, Uterus, *UT P*, Uterine pore, *VD*, Vas deferens, *VG*, Vagina, *VIT*, Vitellaria, *VS*, Vesicula seminalis, *VTR B*, Ventral transverse bar

structure measuring 0.07×0.09 mm, mouth (*M*) situated terminally leading into the pharynx through a buccal cavity (Fig 1, *BC*), oesophagus very short or wanting and bifurcating posteriorly into two simple intestinal cæca (Fig 1, *IC*), each extending posteriorly up to a short distance anterior to the beginning of the haptor. Testis (Fig 1, *T*) single, large, oval, post-ovarian measuring $0.15-0.56$ mm in length and $0.06-0.14$ mm. in width and situated in the posterior half of the body; vas deferens (*VD*) arising

from the anterior end of the testis, passing by the side of the ovary and opening into a laterally placed vesicula seminalis (*VS*), which is very elongate, sigmoid, tubular structure swollen at both ends and situated in the middle of the anterior half of the animal in between the two intestinal cæca, single narrow ductus ejaculatorius (*DE*), originating from the anterior end of the vesicula seminalis and running forwards at the anterior end of the prostatic reservoir (*PR*), where it turns backwards and inwards to form the cirrus (Fig 1, *C*) at its base, a well-differentiated prostate gland (*PR*) divided into two lobes, the terminal end of ductus ejaculatorius (penis) (Fig 5, *P*) elongated, cuticularised tubular structure pointed at its extremity and measuring 0.06 mm in length, and posteriorly with a muscular small rounded penis sac (Fig 5, *PS*), a fine spiral ala (Fig 5, *SA*) with pointed ends running spirally round the organ present on the anterior two-thirds of the penis, usually the penis is sickle-shaped, accessory chitinous, probably hollow spicule (Fig 5, *AC P*) measuring 0.07 mm slightly thicker than the penis, and also a curved body is present, its terminal end slightly spatulate in shape, male genital pore at a distance of 0.4 mm from the anterior end Ovary (Fig 1, *OV*) simple, much smaller than the testis, pear-shaped and situated in the middle of the body, anterior to testis, measuring 0.1-0.30 mm in length and 0.04-0.07 mm in width, oviduct (*OD*) continuous anteriorly with the ovary and continued into the uterus (*UT*) which opens to the exterior (*UT P*) in the region of the anterior end of vesicula seminalis, at a distance of about 0.3 mm from anterior end, a single vagina (*VG*) probably chitinous and opening to the outside, in the region of the transverse vitelline duct, at a distance of 0.7 mm from anterior end, narrow vaginal duct passes from the vagina to the oviduct, the duct functions as receptaculum seminis, transverse vitelline duct (*T VD*) just in front of the conical oviduct, small shell gland (*SG*) opens into the uterus in the region of oviduct Vitellaria (*VIT*) small but well developed extending in the region from pharynx to a distance midway between the testis and the haptor, two rows of about 6-7 pairs of gland cells (*GL C*) in the region between the receptaculum seminis and vagina They have been termed by Yamaguti (1934) as gland cells (*GL C*) Egg was not present in any of the specimens

(b) Sub-Family —Diplectaninae Monticelli, 1903

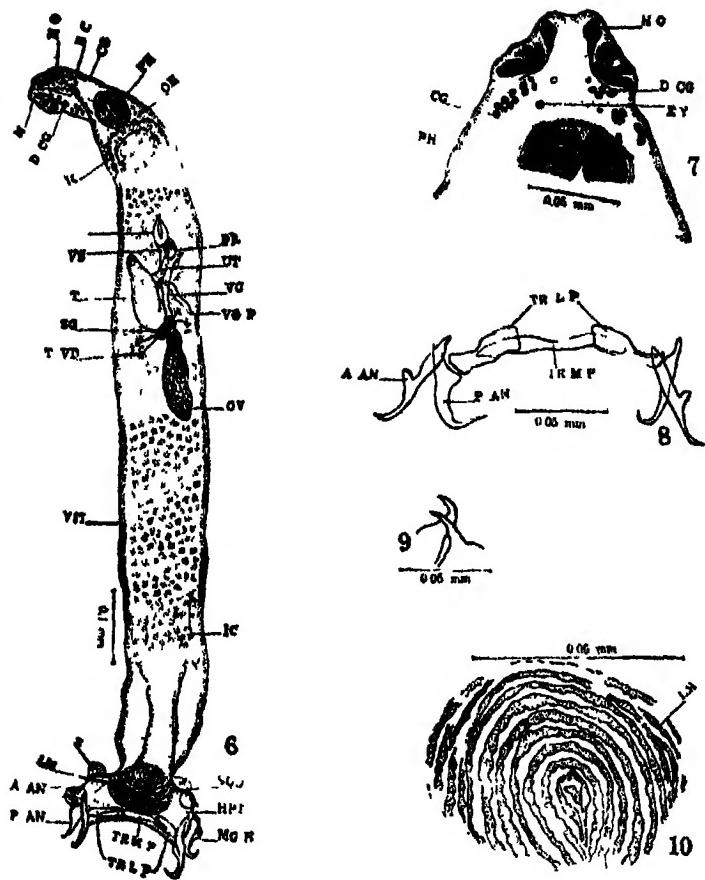
(Syn Lepidotreminae Johnston and Tiegs, 1922

Genus —*Lamellocidiscus* Johnston and Tiegs, 1922

(2) *Lamellocidiscus belengiri*, n.sp.

(Figs 6-10)

Many specimens of this parasite were obtained on the gills of the marine fishes, *Sciæna belengeri*, *Muraenesox talabonoides* and *Sciæna carulta* in



Figs 6-10 *Lamellodiscus belengeri*, n.sp.—Fig. 6 Entire view Fig. 7 Anterior end to show head organs cephalic glands, their ducts and eyes Fig. 8 Dorsal and ventral pairs of anchors with transverse bars Fig. 9 Penis hooks Fig. 10 Squamodisc, showing concentric lamellae

LM, Lamellæ, *OE*, Oesophagus, *S*, Outgrowth on haptor, *SQD*, Squamodisc, *TRLP*, Lateral piece of transverse bar, *TRMP* Median piece of transverse bar *VGP* Vaginal pore, other lettering as in previous figures

November-December 1939 and also in December-January 1940-41 Infestation was not so heavy as in the case of the previous specimen

Shape of the body varies from elongate (Fig. 6) to oval, measuring 0.52-1.17 mm in length and 0.11-0.225 mm in width Cephalic glands (Figs 6 and 7, CG) 8-9 in number, situated in the region of the pharynx and their ducts opening through three pairs of head organs (*HO*); eyes (Fig. 7,

EY) two pairs, the anterior pair smaller; posteriorly, the body terminates in a distinct haptor (Fig 1, *HPT*) measuring 0 11–0 27 mm in diameter, the diameter of the haptor much greater than the width of the animal, haptor with two pairs of hooks (Fig 8), curved distally, one dorsal (Fig 8, *A AN*) and one ventral (Fig 8, *P AN*), average length of an anchor about 0 066 mm ; dorsal pair of anchors biramous and the ventral only slightly so, anchors articulated laterally by means of a single transverse bar, consisting of a central piece (Fig 8, *TR MP*) measuring 0 08 mm and two right and left lateral ones (Fig 8, *TR LP*) measuring about 0 04 mm each, haptor carries three pairs of foliate round, cutaneous outgrowths (Fig 6, *S*) on each side anteriorly, each outgrowth bearing a marginal hooklet (*MGH*), haptor bears a special adhesive disc, squamodisc, consisting of concentric rows of lamellæ (Fig 10, *LM*) made up of continuous chitinous matter, both on dorsal and ventral sides, number of lamellæ varying from 5–16 on each side Mouth (Fig 6, *M*) terminal or subterminal mid-ventral, situated in front of the eyes (*EY*) leading into pharynx (*PH*) through the buccal cavity (*BC*), pharynx muscular, oval, measuring 0 045–0 05 mm, œsophagus practically wanting, two intestinal cæca (Fig 6, *IC*) simple and posteriorly not uniting, terminating much anterior to the haptor Gonads situated in the posterior part of the anterior half of the body, testis (Fig 6, *T*) elongately oval in shape, usually preovarian, situated on the left side, size and shape variable, being 0 045–0 11 mm in length and 0 03–0 04 mm in width, vas deferens arises from the anterior end of the testis leading into a comparatively short vesicula seminalis (*VS*) and opening into the cirrus (*C*), prostate gland (*PR*) posterior to penis, penis enclosed into a sac, a simple structure consisting of two hook-like structures (Fig 8) measuring about 0 026 mm, pointed anteriorly, rather broad and flat posteriorly, arranged like a pair of jaws (Fig. 6, *C*), male genital pore situated posteriorly in the anterior third of body Ovary (*OV*) elongately oval rather tapering anteriorly, lying in the middle of the body posterior to testis or in the neighbourhood of its posterior portion to the right side of the body, measuring 0 10–0 13 mm. in length and 0 03–0 04 mm in width, uterus (*UT*) short and opening just beside the penis, vagina (*VG*) opens about midway between the cirrus and the ovary to the right side (*VG P*), shell gland (*SG*) small and situated near the transverse vitelline duct, vitellaria (*VIT*) extending from the region of pharynx to a distance slightly posterior to the termination of intestinal cæca, transverse vitelline duct (*TV D*) just in front of the ovary No eggs were found.

(B) *Sub-Order* —*Polyopisthocotylea* Odhner, 1912

Super-Family —*Dichidophoroidea* Price, 1936

(i) *Family* —*Dichidophoridae* Fuhrmann, 1928

(Syn *Choricotylidae* Rees and Llewellyn, 1941)

Sub-Family —*Cyclocotylinae* Price, 1943

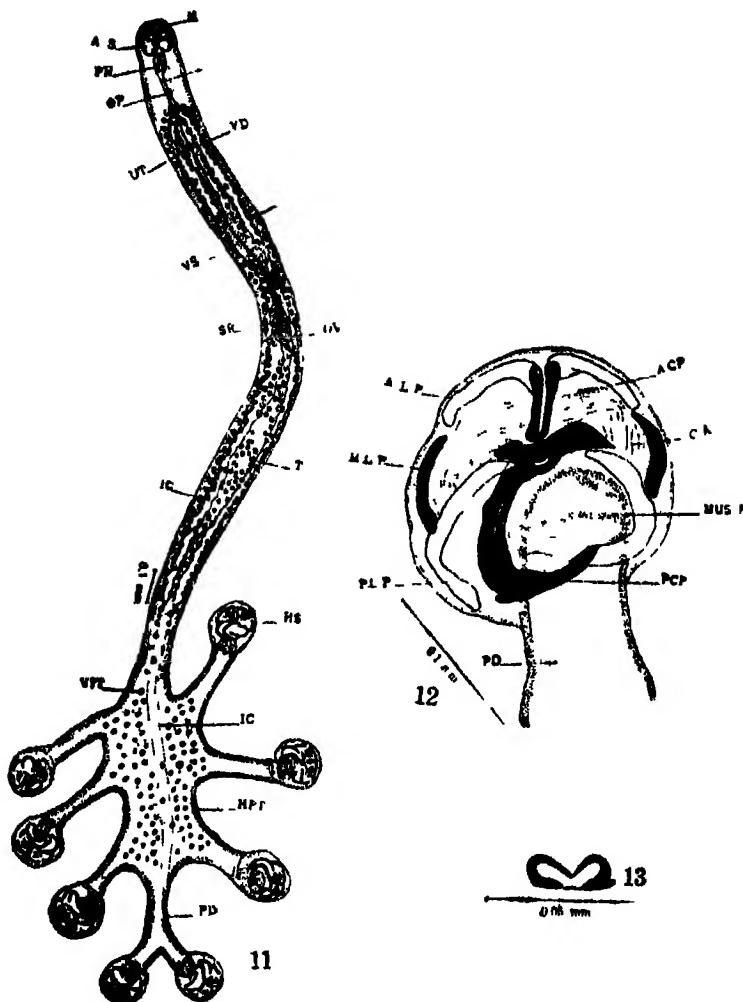
Genus —*Cyclocotyla* Otto, 1823

(3) *Cyclocotyla multatesticulae*, n.sp.

(Figs 11-13)

Only two specimens of this parasite were found in November 1939, on the gills of a marine fish *Pellona* sp

Body elongate (Fig. 11), 2.83 mm long, 0.13 mm wide, anterior end broad and flat, the sides of the body nearly parallel, posterior haptor (*HPT*) palmate, about 0.42 mm in length and 0.25 mm in maximum width, carrying four pairs of pedunculated haptoral suckers (*HS*). Peduncles (*PD*) of suckers long and thick, their length decreasing from before backwards, suckers are "cuplike" (Price, 1943), almost equal in size, average diameter of each 0.12 mm, general structure of the suckers quite typical of the genus, the skeletal elements of each sucker made up of eight chitinised pieces (Fig. 12)—two unpaired central pieces situated one behind the other and three lateral ones on each side, anterior central (*AC P*) U-shaped carrying anteriorly two radiating pieces termed right and left (*4 LP*) anterior lateral pieces; posterior unpaired central piece (*P CP*) large and curved central axis of this piece hollow, laterally this piece articulates with right posterior lateral piece (*P LP*) and left posterior lateral piece (*P LP*) right and left (*M LP*) median lateral pieces attached to the middle of the posterior lateral piece, running anteriorly on each side. Further the surface of inner walls of the posterior central piece (Fig. 12, *P CP*) heavily corrugated on the right side, anterior dorsal space in between the lateral pieces and the anterior and middle piece further supported by two systems of very small chitinous thin rodlets (Fig. 12, *CA*) lying inside the muscular walls, each system consisting of 7-9 concentric arcs, the number of rodlets varies in each case, each sucker with a highly muscular pad (*MUS P*) in the depth of the sucker cavity, languette absent. Mouth (Fig. 11, *M*) subterminal transversely oval, anterior suckers (*AS*) in the form of two muscular and oval openings into the buccal cavity measuring about 0.045 in width and 0.065 mm in length, pharynx (*PH*) muscular, spherical, measuring 0.03 × 0.045 mm and followed by a narrow Oesophagus (*OF*) bifurcating posteriorly to form two main intestinal limbs branched laterally specially on the outside, intestinal cæca uniting posteriorly and running practically to the end of the haptor. Testes (Fig. 1, *T*) about

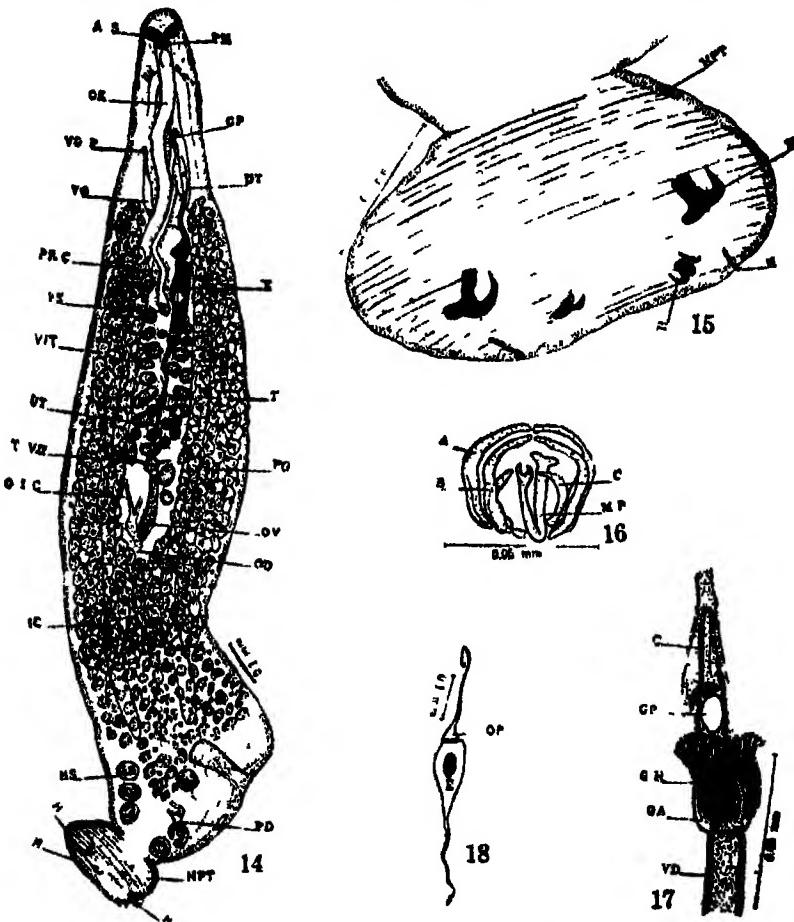


Figs 11-13 *Cyclocotyla multatesticula*, n.sp.—Fig 11 Entire view Fig 12 Haptoral sucker, showing arrangement of the cuticular pieces of its framework Fig 13 Two of the cirrus hooks

ACP, Anterior central piece, *ALP*, Anterior lateral piece, *AS*, Anterior sucker, *CK*, Rodlets of concentric arcs, *GP*, Genital pore, *HS*, Haptoral sucker, *MLP*, Median lateral piece, *MUS P*, Muscle pad, *PCP*, Posterior central piece, *PD*, Peduncle, *PLP*, Posterior lateral piece, *SR*, Receptaculum seminis other lettering as in previous figures

150, small, follicular, situated between the intestinal limbs in the post-ovarian region, vas deferens running anteriorly, ventral to the receptaculum seminis (*SR*) forms anteriorly a vesicula seminalis (*VS*) at the base of the cirrus; cirrus provided anteriorly with a crown of eight inwardly curved

hooks (Fig. 13) the height of each hook being 0.01 mm., genital pore (*GP*) situated halfway between the pharynx and the intestinal bifurcation, on the oesophagus, at a distance of 0.18 mm from the anterior end. Ovary (*OV*), small, median, preequatorial, measuring 0.13 mm. in length, receptaculum seminis (*SR*) massive, preovarian, vitellarian follicles (*VIT*), numerous relatively large, extending from the level slightly below the genital pore to



FIGS 14-18 *Bilaterocotyle chirocentrosus*, n.g. et n.sp.—Fig. 14 Entire view. Fig. 15 Framework of chitinous pieces of haptor sucker. Fig. 16. Framework of chitinous pieces of haptor sucker. Fig. 17 Genital atrium, showing genital spines. Fig. 18 Egg.

A, Outermost, *B*, Middle, *C*, Innermost, and *MP*, Median pieces of the chitinous framework of the haptor sucker, *C'*, Cirrus protruded out, *E*, Egg; *GA*, Genital atrium, *GH*, Genital hooks, *GIC*, Genito-intestinal canal, *H*, *H'*, *H''*, Haptoral hooks of the first, second, and third pair respectively, *OP*, Operculum; *PG*, Pigment granules, *PR C*, Prostate gland cells; other lettering as in previous figures.

posterior end of the body and extending into the haptor. No egg was observed.

(ii) Family—Microcotylidæ Taschenberg, 1879

Sub-Family—Protomicrocotylinæ Johnston and Tiegs, 1922

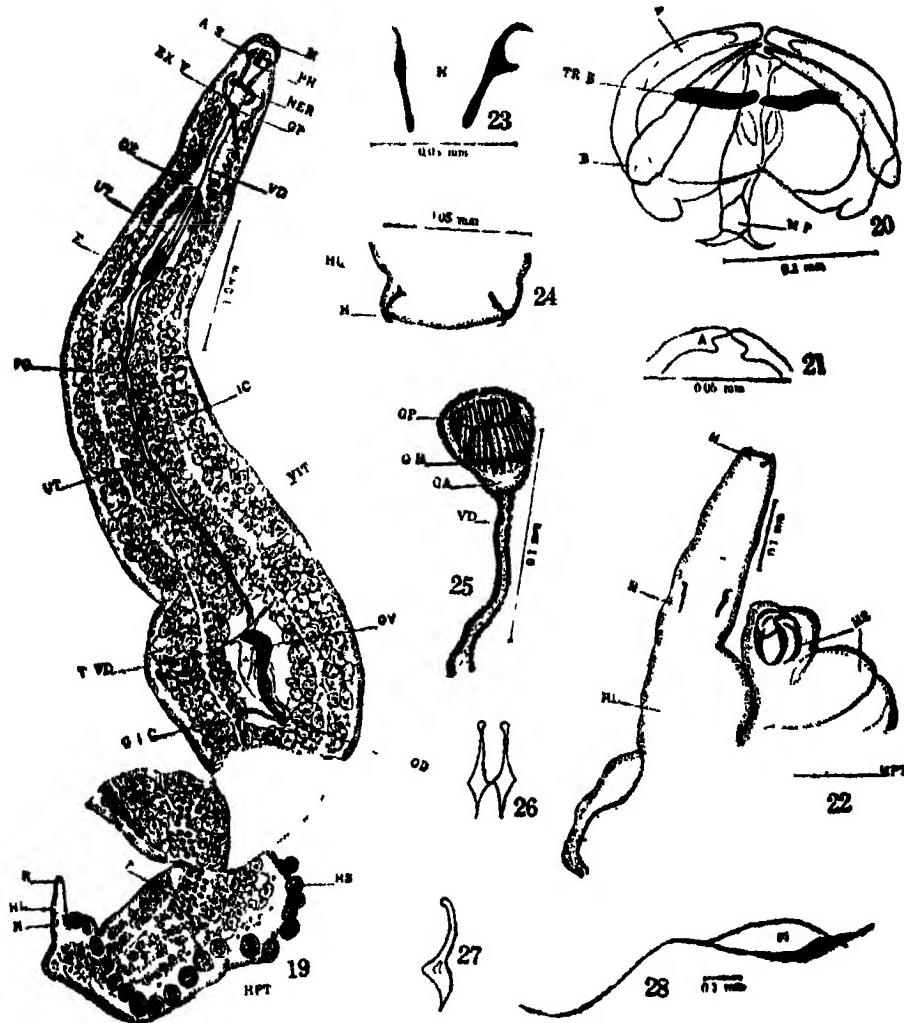
Genus—*Bilateracotyle* n.g.

(4) *Bilateracotyle chirocentrosus*, n.g. et n.sp.

(Figs 14–18)

Seven specimens of this parasite were obtained in November 1939 on the gills of a marine fish *Sciaena helengeri* and only one in December 1939 from the gills of *Chirocentrus dorab*. Forms obtained from both the hosts are identical except in unessential respects.

Body elongate (Fig. 14), flat, tapering anteriorly, broadest in the middle and tapering into a distinctly marked off disc, haptor (*HPT*) bearing usually three pairs of hooks (*H H' H''*), posterior end of the body proper bears three pairs of retractile pedunculated (Fig. 14, *PD*) chitinous suckers in two longitudinal rows immediately before the commencement of the disc, body length of the worm 2.05 mm maximum width 0.41 mm, structure of the framework of the chitinous pieces of the sucker (Fig. 14 *HS*, Fig. 16) typically microcotylid, each sucker supported by a framework of three pairs of lateral pieces (*A, B, C'*) on each side and one bent median piece (*MP*), two of the outer lateral pieces long and strongly recurved almost meeting distally, the long and median piece bent upon itself and presenting somewhat the appearance of the letter U, average diameter of a sucker is 0.04 mm, the orientation of these pieces, with reference to the axis of the body, in various suckers variable, posterior haptoral disc, oval in shape, measuring 0.24 × 0.13 mm with transverse muscular striations, outer pair of disc hooks (Fig. 15, *H*) largest and anchor-shaped, measuring 0.05 mm in length, the second pair (*H'*) more posterior, rather towards the margin, long and thin, situated laterally in between the space of the outer and inner pair, measuring 0.02 mm in length, inner pair of hooks (*H''*) more or less similar in shape to the outer, though smaller, measuring 0.026 mm in length. Anterior suckers (Fig. 14, *AS*) two, elongately oval with membranous septa, measuring 0.04 × 0.02 mm, pharynx (*PH*) bulb-shaped, Oesophagus (*OE*) very long, slightly sinuous, bifurcating into two intestinal cæca (*IC*) with ramifying branches laterally and terminating just anterior to the beginning of the posterior suckers. Testes (Fig. 14, *T*), follicular, 20–28 in number and situated in the middle third of the body, in between the cæca, preovarian, the very much coiled vas deferens opening into vesicula seminalis (*VS*) which is very long, sinuous tubular running anteriorly in midline and surrounded on all sides



Figs 19-28 *Pseudaxine indicana*, n.sp.—Fig. 19 Entire view Fig. 20 Framework supporting the haptoral sucker Fig. 21 Outermost piece of Fig. 20, lateral view Fig. 22 Haptoral languette ("Proboscis"), showing the arrangement of two pairs of hooks Fig. 23 Second pair of haptoral hooks, situated in the middle length of the proboscis Fig. 24 Terminal portion of proboscis showing the first pair of haptoral hooks Fig. 25 Genital atrium, showing the genital hooks and the genital pore Fig. 26 Genital hooks enlarged Fig. 27 Genital hook lateral view Fig. 28 Egg

EX V, Excretory vessel. *HL*, Haptoral languette. *NER*, Part of nervous system, other lettering as in previous figures

by well-developed prostatic cells (*PR C*), opening in the genital pore (*GP*) through a long tubular unarmed cirrus (Fig. 17, *C'*); it begins just anterior

to the anterior testis and has a slightly swollen base filled with sperms, genital pore to the right side, midway between the pharynx and the intestinal bifurcation, at a distance of 0.26 mm from the anterior end; genital atrium (Fig. 17 *GA*) elliptical, large and armed by a coronet of long chitinous 24–38 spines (Fig. 17 *GH*) average length of spines being 0.02 mm. Ovary (Fig. 14, *OV*) 0.17 × 0.03 mm, median elongated, situated in the anterior portion of the posterior half of the body. genito-intestinal canal (Fig. 14, *GIC*) opens into the left cæcum of the intestine, transverse vitelline ducts (*TVD*) just anterior to the ovary, vitellaria (*VIT*) large, follicular, extending from the point of intestinal bifurcation upto the haptorial suckers, usually following the course of intestinal cæca specially in the region, anterior to the ovary, pigment granules (*PG*) scattered amongst vitelline follicles, uterus (*UT*), median visible up to the genital atrium, vagina (*VG*) a simple tube, opening laterally to the left side, the opening (*VG P*) situated at a level slightly lower than the genital pore. Only one operculate egg (Fig. 18) in the uterus, spindle-shaped with polar filaments, measuring 0.23 mm (without filaments) in length and 0.04 mm in width.

(iii) Family—*Gastrocotylidæ* Price, 1943

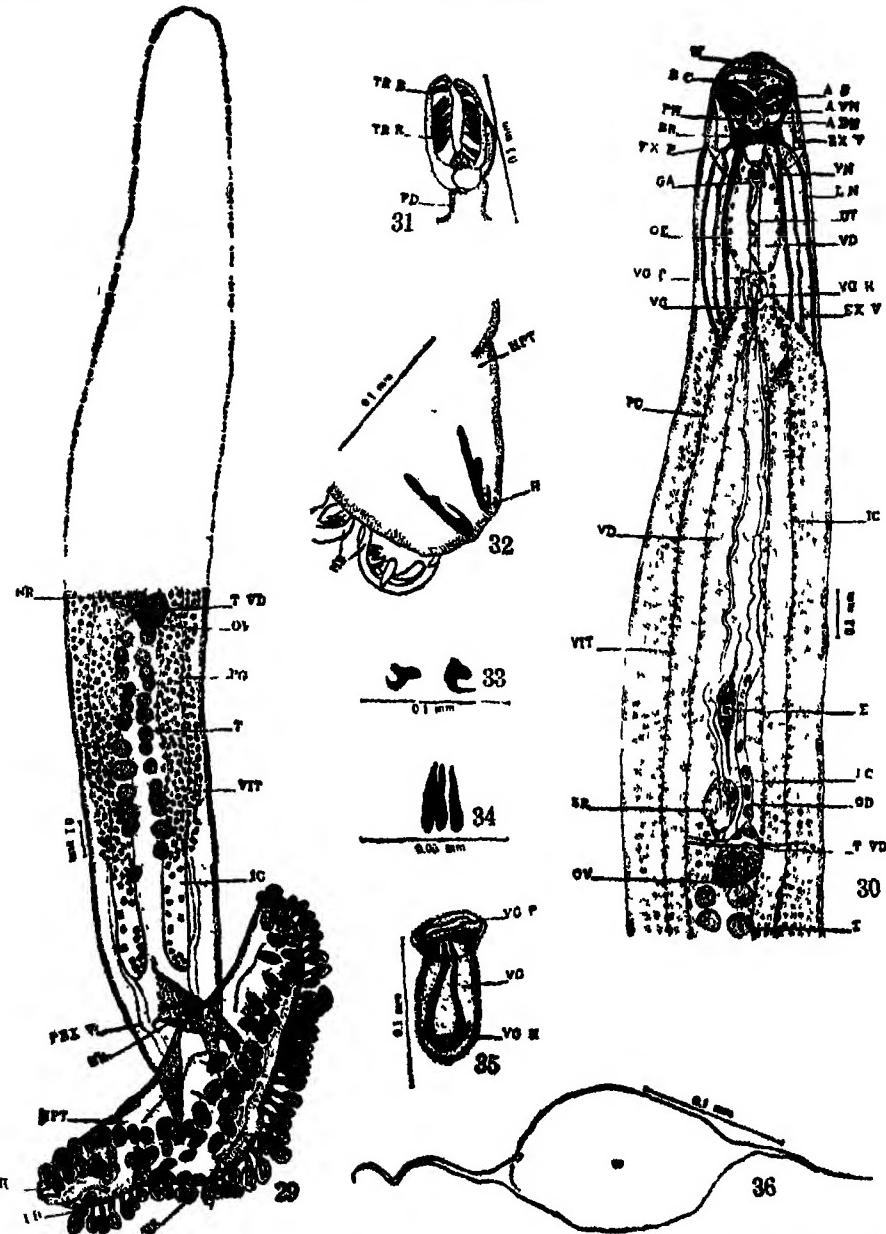
(a) Genus—*Pseudoxine* Parona and Perugia, 1890

(5) *Pseudoxine indicana*, n sp

(Figs 19–28)

A single good specimen of this very interesting form was obtained in January 1941 on the gills of marine fish *Chrysophrys berda*

Body elongate (Fig. 19) tapering anteriorly, broad posteriorly measuring 9.16 mm in length and 1.6 mm in maximum width at the level of the ovary, posteriorly fan-shaped cotylophore (= haptor) (*HPT*) inclined to the body and separated from it by a notch, carrying 19 suckers, in a single row, on its lower margin, average diameter of a sucker 0.16 mm, framework of cuticular pieces (Fig. 20) consisting of two pairs of lateral pieces (*AB*), the outer of which bifid at both ends, a median piece (*MP*) and a pair of transverse pieces (*TR B*), the median piece complex in shape, the extreme of the cotylophore carries an elongated proboscis-like process (Figs 19 and 22, *HL*) measuring 0.5 mm in length and 0.1 mm in width, bearing in the middle of its length a pair of hooks (Figs 22 and 23, *H'*), 0.04 mm. in length and another pair (Figs 22 and 24, *H*) of smaller hooks measuring 0.01 mm in length at its tip. Mouth (Fig. 19, *M*) opening at the anterior end of the body, in which lie two paired egg-shaped anterior suckers (*AS*); mouth leading into the oval pharynx (*PH*) lying between the suckers; Oesophagus (*OE*) about 1.0 mm. in length and bifurcating into two



Figs 29-36 *Pricea mulie*, n.g. et n.sp.—Fig. 29 Posterior half of the parasite Fig 30 Only anterior half of the parasite, Fig 31 Haptoral sucker, lateral view Fig 32 Extreme lateral side of the haptor, showing haptoral hooks. Fig 33 Body hooks Fig 34 Three cirrus hooks Fig. 35 Terminal portion of vagina, showing the vaginal hook and the vaginal opening Fig 36 Egg

A DN, Anterior dorsal nerve, AVN, Anterior ventral nerve, BH, Body hook, BR Brain, EXP, Excretory pore; LC, Laurer's canal, LN, Lateral nerve; PEX V, Posterior excretory vessel; TR R, Transverse rib of the framework of the haptoral sucker, VG H, Vaginal hook; VN, Ventral nerve; other lettering as in previous figures.

intestinal canals (*IC*) sending many lateral branches ramifying specially on the outer side, into the vitellaria, cæca not contiguous posteriorly and running upto near the posterior end of the cotylophore. Testes (Fig 19, *T*) small, follicular, about 40 in number lying irregularly in the inter-cæcal field anteriorly in two rows and posteriorly in three rows, post-ovarian, a few extending into the haptor, vas deferens (*VD*) running anteriorly to the genital opening (*GP*), genital pore on the oesophagus halfway between the pharynx and the intestinal bifurcation, 0.58 mm. from the anterior end of the body armed with a coronet of 24 hooks (Fig 25, *GH*) each of which about 0.022 mm in length, each hook having a curved anterior extremity, a broad body in contact with its neighbour (Figs 26 and 27) Ovary (Fig 19, *OV*), elongate, cylindrical, situated in the median line, in the posterior half of the body measuring 0.77 mm in length and 0.13 mm in width; oviduct (*OD*) arising posteriorly from ovary, receiving the genito-intestinal canal (*GIC*) and the yolk duct (*YD*) and passing on as ootype, uterus (*UT*) running forward to the genital pore; transverse vitelline ducts (*TV*) just anterior to the ovary, vitellaria (*VIT*) on both sides of the body from the level of the genital pore to the end of the cotylophore, black pigment granules (Fig 19, *PG*) few, scattered in vitellaria, single uterine egg with polar filaments, 0.3 mm long (length without polar filaments) and 0.08 mm wide

(b) Genus —*Pricea*, n.g.

(6) *Pricea multae*, n.g. et n.sp.

(Figs 29–36)

Only one good matured specimen of this species was collected from the gills of *Cybum lanceolatus* in December 1939

Body elongately cylindrical (Figs 29 and 30) slightly tapering anteriorly and bearing comparatively a very broad haptor (Fig 29, *HPT*) posteriorly, sides running almost paralleled for most part of the body length, 3.22 mm (with haptor) long and 0.4 mm broad, posterior haptor very broad measuring 1.02 mm in length and 0.33 mm in width, very distinctly set off from the body proper and carrying a pair of recurved haptoral hooks (Figs 29 and 32, *H*) with double roots, at one of its extreme side end Each measuring 0.08 mm in length; the haptor is an elongately oval structure whose long axis is at right angles to the long axis of the body, and usually folded upon itself giving Napoleon's helmet-like appearance, carrying 122 retractile, pedunculated suckers (Figs 29 and 31, *HS*) arranged in two rows, at both margins of its sides, each measuring about 0.07 mm. in diameter, frame work of cuticular structural pieces forming the supporting skeleton of the

haptoral sucker very interesting consisting of a pair of two (Fig 38 A, B) thin, long bars, recurved upon themselves and almost meeting in the middle, a three pronged central piece (*MP*) on a basal piece (*BP*), a pair of transverse bars (*TR B*) contained within the bent extremity of the inner lateral piece, followed by five to seven thinner transverse ribs (*TRR*) and in addition, a pair of lateral pieces each outside the three pronged median one, giving support to the thin transverse ribs (Fig 38, D), a pair of recurved hooks (Fig 29 and 33, *BH*) with double roots, situated in the posterior end of the body, just above the haptor, measuring about 0.03 mm in length Mouth (Fig 30, *M*) sub-terminal leading to a very small globular pharynx (*PH*) through a buccal cavity (*BC*), anterior suckers (*AS*) bilocular, egg-shaped highly muscular organs, with membranous septa, measuring 0.09×0.05 mm, oesophagus (*OE*) elongate, club-shaped and bifurcating into intestinal crura (Fig 29, *IC*) terminating just a little before the origin of haptor, with ramifying branches, laterally, into the vitellaria, which are more numerous on the outer side Testes (Fig 29, *T*) numerous, 26 in number, post-ovarian, follicular, situated in two lateral rows, in between the intestinal crura, size variable, 0.03–0.06 mm in diameter, vas deferens (*VD*) runs anteriorly, in the middle, its course more sinuous in the anterior region, opening into an unarmed genital atrium (*GA*) situated in the midline, on the oesophagus, at a distance of 0.23 mm from the anterior end—through a long muscular armed cylindrical cirrus, with 12 small club-shaped chitinous spines (Fig 34, *CH*) broad at the base and tapering anteriorly Ovary (Fig 30, *OV*) spherical, situated in the middle of the body, measuring 0.11 mm in length and 0.08 mm in width, genito-intestinal canal (Fig 30, *GIC*) present, transverse vitelline yolk ducts (*TV D*) anterior to the ovary, receptaculum seminis (*SR*) big, oval and situated slightly anterior to ovary, on the left side, uterus (*UT*) with a single uterine spindle-shaped egg (Fig 30 and 36, *E*) with polar filaments; oviduct (*OD*) very elongated, leading into the vagina (Fig 30 and 35, *VG*) situated just on the point of bifurcation of the oesophagus into intestinal limbs, at a distance of 0.44 mm from the anterior end; its terminal end dilating to form oval vaginal pouch (*VG P*), containing a doubly curved chitinised U-shaped hook (Fig 35, *VG H*) one of its arms measuring 0.035 mm in length, vaginal opening with fleshy, muscular flaps, presenting the appearance of a rudimentary pseudo-genital sucker (Fig 35), vitellaria (*VIT*) follicular, extending from the level of the genital pore upto the extent of the testes, though a few follicles extending posteriorly, on the intestinal cæca, upto the point of their termination, pigment granules (Fig 29, *PG*) few.

The nervous system (Fig 30) consists of a brain (*BR*) arched over the oesophagus, posterior to pharynx, from its lower end arise the ventral nerve

chords (*VN*) running posteriorly along the sides of the digestive system. Dorsal and lateral to the ventral trunks are the lateral nerve trunks (*LN*) running along the margins of the body. Antero-ventral nerves (*AVN*) supporting the ventral and lateral walls of the oral suckers originating at the junction of the lateral nerves with the brain, run anteriorly on the outer sides of the suckers and complete the ring anteriorly. Near the top of the brain is a pair of antero-dorsal nerves (*ADN*), running along side the oesophagus and pharynx and supplying the dorsal walls of the suckers.

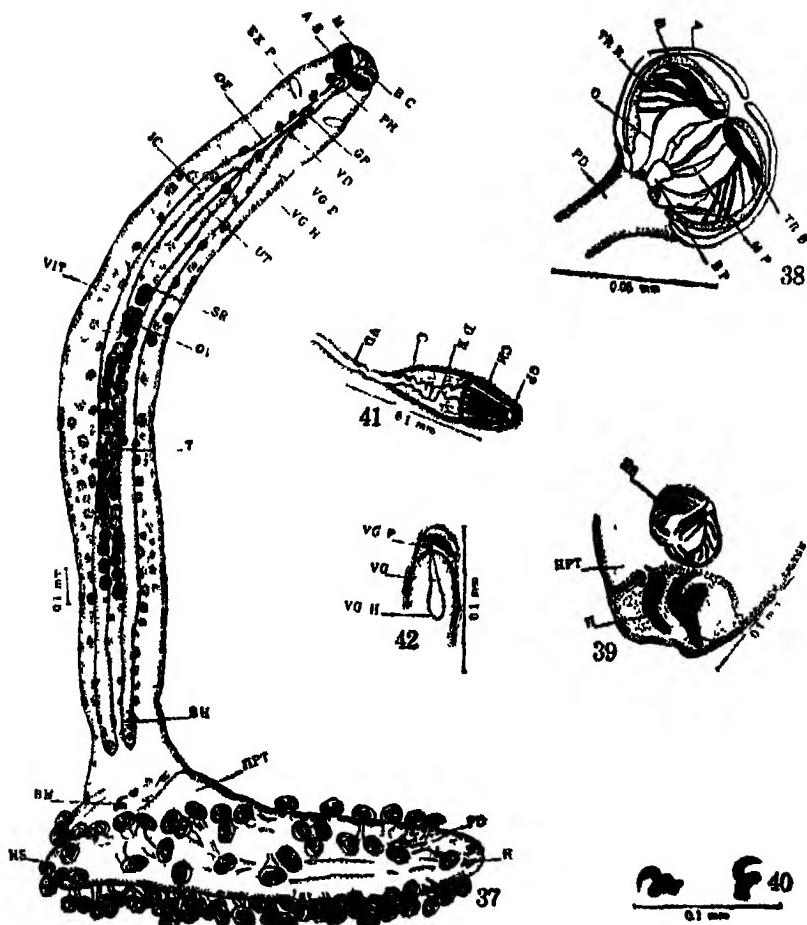
The excretory system (Fig. 30) opens to the exterior through two dorsal and laterally placed spindle-shaped pores (*EXP*) situated laterally at the level of genital pore. An excretory vessel (*EXV*) is seen to run anteriorly and one posteriorly (*EXV*) from both pores, on both sides. The posterior excretory vessels (Fig. 29, *P EXV*) are visible in stained preparation on the outer sides of the intestinal limbs after the termination of the vitellaria into the posterior region, upto the haptor.

(7) *Pricesa minimae*, n.sp

(Figs 37-48)

Six specimens of this fluke were found on the gills of *Thynus relamys*.

Body elongately cylindrical (Fig. 37) with sides almost parallel, anterior end truncated, posterior ending in a foot-like haptor (*HPT*), the entire haptor has the same relation to the body as a foot has to the leg, body length 3.46 mm and maximum width, in the region of gonads, 0.27 mm; two hooks (Figs 37 and 41, *BH*), one behind the other on the posterior end of the body proper, one hook situated on the right intestinal cæcum, near its posterior end and the other just anterior to the haptor slightly to the left, average length of a hook being 0.04 mm, one more pair of recurved hooks (Figs 37 and 39, *H*) situated at one side extremity of the haptor, measuring 0.06 mm, haptor bears, 70 retractile, pedunculated suckers (*HS*) in two rows, along its both sides, with the structure (Fig. 38) of the chitinous framework as described for the genus except the number of ribs which is 5-7, oval in shape with an average diameter of 0.03-0.07 mm., haptor itself measures 0.12 × 0.033 mm. Mouth (Fig. 37, *M*), subterminal leading into the pharynx (*PH*) through a buccal cavity, two anterior suckers (*AS*) elongately oval measuring 0.04-0.07 mm, pharynx small, spherical and muscular organ, oesophagus (*OE*) thin and long, bifurcating into two intestinal cæca (*IC*) extending posteriorly upto just anterior to the haptor. Testes (Fig. 37, *T*) follicular, 28 in number, post-ovarian, and intercæcal, with an average diameter of each 0.02-0.04 mm; male genital duct (Fig. 41, *DE*) opens as usual into the genital atrium (*GA*) through a cirrus.



Figs 37-42 *P. minima*, n.sp.—Fig. 37 Entire view Fig. 38 Haptoral sucker, showing the arrangement of the cuticular pieces of the framework supporting it Fig. 39 Extreme lateral side of the haptor showing haptoral hooks Fig. 40 Body hook. Fig. 41. Cirrus showing the genital pore, arrangement and shape of cirrus hooks, and ductus ejaculatorius Fig. 42 Terminal part of vagina, showing its opening and the vaginal hook

B.P. Basal piece supporting the three pronged middle piece of the cuticular framework supporting the haptoral sucker, **CH**, Cirrus hook, **D**, Innermost thin and lameilar lateral piece of the framework of the haptoral sucker, **DE**, Ductus ejaculatorius, other lettering as in previous figures

(Fig. 41. C) very much coiled, cirrus elongated, cylindrical and muscular organ, armed with 10 spines, each measuring 0.03 mm; genital pore (GP) situated, in the midline, on the oesophagus at a distance of 0.21 mm from the anterior end Ovary (OV) pear-shaped, pretesticular,

situated in the middle of the body measuring $0\ 05 \times 0\ 08$ mm , receptaculum seminis (*SR*) smaller than the ovary and preovarian, vagina (*VG*) with the characteristic-U shaped hook (Fig 42, *VG H*) one of its arm measuring $0\ 045$ mm , situated on the oesophagus at the point of its bifurcation at a distance of $0\ 4$ mm from the anterior end; vitellaria (*VIT*) very few, follicular, extending mostly from the level immediately below the vagina to the end of the intestinal limbs, few scattered follicles anterior to vagina Uterus and egg could not be observed Two excretory pores (Fig 37, *EX P*) each placed laterally at the level of the genital pore, along the margins of the body

Host — *Thynus pelamys*

Location — Gills

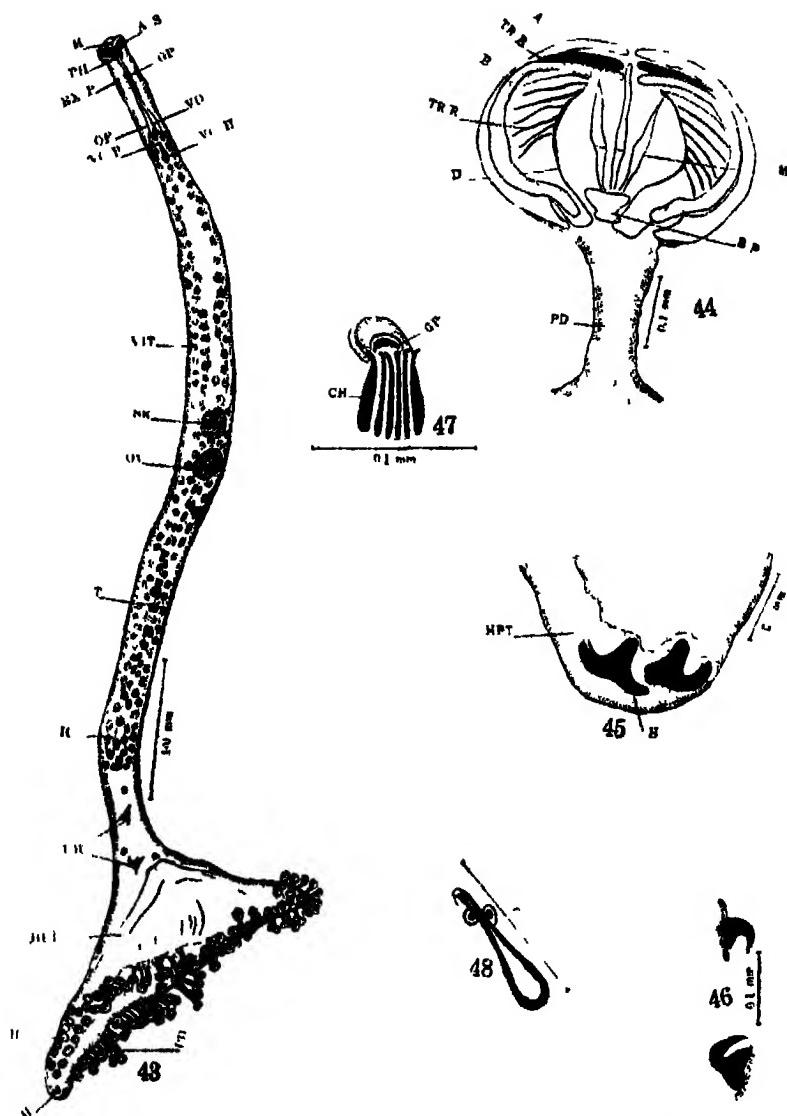
Locality — West coast of India (Bomby)

(8) *Pricea microcotylae*, n.sp

(Figs 43-48)

About a dozen specimens of this fluke were found on the gills of *Scomber microlepidotus*

Body very narrow (Fig 43) thin and elongate, measuring 70 mm. in length and 0.37 mm in maximum width (in the region of gonads), posteriorly ending in an asymmetrically situated haptor (*HPT*) carrying 113 suckers, with retractile peduncles (*PD*); suckers (*HS*) comparatively smaller in size and arranged in two rows on both sides, average measurement being $0\ 026 \times 0\ 078$ mm , four hooks, one pair (Figs 43 and 45, *BH*) situated at one end of the haptor, measuring 0.11 mm in length (average); the two others (Figs 43 and 46, *BH*) situated one behind the other at the posterior end of the body proper, one not on the right intestinal cæcum as in the last species, average length is 0.08 mm , framework of the suckers (Fig. 44) characteristic of the genus, except for the number of the ribs which is 5-6 Mouth (*M*) subterminal leading into a small bulbous pharynx (*PH*), two anterior suckers (*AS*) oval, measuring $0\ 05 \times 0\ 10$ mm ; oesophagus (*OE*) long and very thin, two limbs of the intestine (*IC*) terminating anterior to the body hook and vitellaria Testes (Fig. 43, *T*) very small, follicular, arranged into two irregular rows, posterior to the ovary, about 25,in number, vas deferens (*VD*) opening into the genital pore (*GP*) situated on the oesophagus at a distance of 0.26 mm from the anterior end; number of cirrus hooks (Fig 47) 12 each slightly recurved on its outer side in this species and measuring 0.05 mm in length (average). Ovary (*OV*) median, spherical, measuring $0\ 156 \times 0\ 234$ mm and pretesticular, receptaculum seminis



Figs 43-48 *P. microcotyle*, n. sp.—Fig. 43 Entire view Fig. 44 Haptoral sucker showing the arrangement of the cuticular pieces of its framework Fig. 45 Extreme side of the haptor showing the two haptoral hooks Fig. 46 Body hook Fig. 47 Cirrus hook, showing their arrangement in the currus Fig. 48 Vaginal hook

Lettering as in previous figures

(SR) slightly bigger than ovary, situated anterior to it; uterus (UT) seen only in the region anterior to the vagina; vaginal pore situated on the intestinal

bifurcation, at a distance of 0.8 mm from the anterior end, vaginal hook (Fig. VG H) comparatively larger, thin, its one arm measuring 0.08 mm, vitellaria (VIT) extending from the region anterior to the vaginal pore up to a distance a little posterior to the end of the intestinal crura. No egg was observed. Two lateral, excretory pores along the margin, at the level of the genital opening.

Host—*Scomber microlepidotus*

Location—*Gills*

Locality—West coast of India (Bombay)

IV DISCUSSION

Odhner (1912) subdivided the group *Monogenea* into two sub-orders, *Monopisthocotylea* and *Polyopisthocotylea* on the basis of the presence of a genito-intestinal canal in the former and its absence in the latter. Monopisthocotylids have a single posterior organ of attachment and Polyopisthocotylids have many. Fuhrmann (1928) divided the sub-order *Monopisthocotylea* into two sub-orders *Monopisthodiscinea* and *Monopisthocotylinea*. Price (1936) retained Odhner's classification but divided Odhner's sub-order *Monopisthocotylea* into two super-families, *Gyrodactyoidea* and *Capsaloidea*, and the sub-order *Polyopisthocotylea* into two super-families, *Polystomatoidae* and *Diclidophoroidea*.

The superfamily *Gyrodactyoidea* was created by Johnston and Teigs in 1922 and can be distinguished from the super-family *Capsaloidea* Price, 1936 by the possession of transverse supporting bars to the hooks in the haptor, the haptor of forms belonging to the super-family *Capsaloidea* being either unarmed or if armed lack the supporting bars.

The super-family *Gyrodactyoidea* contains four families which can be distinguished one from the other by the key given by Price (1937). I have described in this paper two new species belonging to one of the four families only, viz., *Dactylogyridæ* Bychowsky, 1933. This family contains four sub-families, viz., *Bothitrematina* Price, 1936, *Dactylogyrina* Bychowsky, 1933, *Diplectanina* Monticelli, 1903 and *Tetraonchinæ* Monticelli, 1903. A useful key to these sub-families is also given by Price (1937).

The new species *Ancylocephalus alatus*, belongs to the sub-family *Tetraonchinæ* because its haptor is without accessory structures or squamo-discs. Price (1937) gives a key to the genera of this sub-family. He includes twenty genera in his key and puts *Dactylogidus* Olsson, 1893 as a genus inquirenda. He included all the nine new genera of Mueller in his key only provisionally. Subsequent to Price's monograph following additional genera

have been added to the family — *Ancylodiscoides* Yamaguti, 1937, *Ancyrocephaloides* Yamaguti, 1938, *Parancyrocephaloides* Yamaguti, 1938 and *Anchoradiscus* Mizelle, 1941. Of the nine genera of Mueller only three, viz., *Cleidodiscus*, *Urocleidus* and *Actinocleidus*, have been retained in this sub-family by Mizelle and Hughes (1938) and Seamster (1938) who have given very acceptable reasons for regarding the others as synonyms of some of these three genera. In view of these additions and alterations, I give below a new key to the genera of this sub-family.

Key to the genera of the sub-family Tetraonchinae --

1	One pair of head organs	<i>Diplectanotrema</i> Johnston and Tiegs, 1922
	More than one pair of head organs	2
2	Intestine single	<i>Tetraonchus</i> Diesing, 1858
	Intestine double	3
3.	Intestine uniting posteriorly	4
	Intestine not uniting posteriorly	13
4	Eyes absent	<i>Tetraoncistrum</i> Goto and Kikuchi, 1917
	Eyes present	5
5.	Vitellaria not extending into posterior third of body	6
	Vitellaria extending into posterior third of body	7
6	Vagina present	<i>Daireosoma</i> Johnston and Tiegs, 1922
	Vagina absent	<i>Empleurosoma</i> Johnston and Tiegs, 1922
7.	Haptor not disc-like	8
	Haptor disc-like	10
8	Anchors with 2 pairs of non-articulating haptoral bars	12
	Anchors without or with only one pair of supporting bars	9
9	Anchors totally without supporting bars, one pair of eyes, testis oval without incision	<i>Ancyrocephaloides</i> Yamaguti, 1938
	Anchors with saddle-shaped supporting bar only between the ventral pair of anchors, two pairs of eye-spots, testis deeply incised appearing as if folded upon itself	<i>Parancyrocephaloides</i> Yamaguti, 1938

10.	Enormously developed, ovate, anchor bases, anchor shafts vestigial or wanting Very small anchor bases, anchor shafts present	<i>Anchoradiscus</i> Mizelle, 1941 11
11	Marginal hooklets 14, posterior disc four lobed, anchors without an accessory piece Marginal hooklets reduced, posterior disc four lobed : all anchors with an accessory piece	<i>Actinocleidus</i> Mueller, 1937 <i>Anchelodiscoides</i> Yamaguti, 1937
12	Vagina absent Vagina present	<i>Urocleidus</i> Mueller, 1934 <i>Cleiodiscus</i> Mueller, 1934
13	Eyes present Eyes absent	14 16
14	Vagina absent Vagina present	<i>Anchyodiscus</i> Johnston and Tiegs, 1922 15
15	Vaginal aperture median, haptor with three bars Vaginal aperture lateral, haptor with two bars	<i>Murraytrema</i> Price, 1937 <i>Ancyrocephalus</i> Creplin, 1839
16	Haptor without bars Haptor with 1 or 2 bars	<i>Amphibdella</i> Chatin, 1874 17
17	Haptor with one bar Haptor with two bars	<i>Amphibdelloides</i> Price, 1937 <i>Haliotrema</i> Johnston and Tiegs, 1922

The species, *Ancyrocephalus alatus*, n sp possesses three pairs of head organs, large hooks supported by cuticular bars, only twelve marginal hooklets, intestinal cæca not uniting posteriorly, two pairs of eyes and the vitellaria extending into the posterior third of the body. In view of these characters, the form belongs to the genus *Ancyrocephalus* Creplin, 1939. Creplin created this genus in 1939 and named *A. paradoxus* as the type species. Johnston and Tiegs (1922) recognised twelve species belonging to this genus. Price (1937) reviews the species and considers that the following ten belong to this genus.—*A. paradoxus* Creplin, 1839 (type species), *A. atherina* Price, 1934; *A. bassensis* Hughes, 1928; *A. lactophrys* (MacCallum, 1915), Johnston and Tiegs, 1922; *A. manilensis* Tubangui, 1931, *A. similis* Price, 1937; *A. teuthis* (MacCallum, 1915) Johnston and Tiegs, 1922, *A. tylosuri* (MacCallum, 1917), Johnston and Tiegs, 1922; *A. vanbenedenii* (Parona and Perugia, 1890) and *A. vesiculosus* Marrey 1931.

Since then the following additional new species have been added to the genus.—*A thysanophrydis* Yamaguti, 1937, *A lethrinii* Yamaguti, 1937 and *A. parvus* Linton, 1940

The species *Ancyrocephalus alatus*, n sp differs from all the known species of the genus in the general shape of the body, the number of marginal hooklets which is only twelve, the structure of vesicula seminalis and penis possessing a spiral ala and the shape of the accessory piece

Lamellodiscus belengeri, n sp belongs to the sub-family *Diplectaninae* Monticelli, 1903. The characteristic feature of this sub-family is the presence of a pair of dorsal and ventral accessory structures known as squamodiscs on the haptor. The sub-family contains three genera *Lamellodiscus* Johnston and Tiegs, 1922, *Lepidotrema* Johnston and Tiegs, 1922 and *Dilectanum* Diesing, 1858. Price (1937) has given key to the three genera. The squamodiscs of the genus *Lamellodiscus* show concentric rows of paired lamellæ and have three cuticular bars supporting the anchors. The genus was created by Johnston and Tiegs, 1922 with *L. typicus* as the type species. Since then only two species *L. pagrosomi* Murray, 1931 and *L. major* Murray, 1931 have been added to this genus. The new species differs from all the known species of the genus in the possession of the peculiar six outgrowths on the haptor, in the number of marginal hooks being only six, in the peculiar but simple structure of the penis which has only two simple hooks and in the pre-ovarian position of the testis. The vagina is also without chitin, also the lamellæ of the squamodisc are continuous.

Included in the sub-order Polyopisthocotylea are the two super-families Polystomatoidea Price, 1936 and Diclidophoroidea Price, 1936. I have described in this paper six forms all of which belong to the super-family Diclidophoroidea. This super-family is distinguished from Polystomatoidea, the other super-family of this sub-order, by the presence of a pair of small suckers on the anterior end of the body. The super-family contains six families: *Diclidophoridae* Fuhrmann, 1928, *Discocotylidae* Price, 1936, *Mazocraeidae* Price, 1936, *Hexostomatidae* Price, 1936; *Gastrocotylidae* Price, 1943 and *Microcotylidae* Taschenberg, 1879. Price (1943) gives a provisional key to distinguish the families. His classification is based mainly on the number and shape of the cuticular pieces composing the framework of the haptoral suckers. Price himself is not satisfied with this basis. The difficulty is further aggravated by the fact that no suitable descriptive terms have been proposed for these structures. Price (1943) has given diagrams to show the types of frameworks of the haptoral suckers in these six families. I have used Price's key and his diagrams to distinguish the new forms.

The family *Diclidophoridae* Fuhrmann, 1928 has eight principal cuticular pieces in the framework of the haptoral sucker (see Price Fig 1, page 45 1943) The family contains two sub-families *Diclidophorinae* Cervantaine, 1895 and *Cyclocotylinae* Price, 1943 The former has "clamp-like" or "pincer-like" sucker and in the latter, the sucker is "cup-like" Price (1943) has given a key to the seven genera comprised within the sub-family *Cyclocotylinae*

Cyclocotyla multatesticula n sp described in this paper belongs to the genus *Cyclocotyla* Otto, 1823 because the haptor is distinctly set off from the body, vitellaria extend into the haptor, vagina is absent, testes are post-ovarial and cirrus is armed A complete list of species of this genus is given by Price (1943) but not a key

I give below a key to the species of this genus including *C. multatesticula*, n sp

Key to the species of the genus Cyclocotyla —

- | | |
|-------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| 1 Vaginae two, opening in the neighbourhood of the genital atrium | <i>C. tachebnergi</i> (Parona and Perugia, 1889) Price, 1943 |
| Vaginae absent | 2 |
| 2 Body proper (not including the haptor) clearly divisible into two regions | 3 |
| Body proper not divisible into two regions | 4 |
| 3 Anterior part of body demarcated from posterior by distinct shoulders | <i>C. bellones</i> Otto, 1823 (Type species) |
| Two regions of the body merge imperceptibly into one another | <i>C. charcoti</i> (Dollfus, 1922) Price, 1943 |
| 4 Peduncles of unequal length | <i>C. smaris</i> (Iijima, in Goto, 1894) Price, 1943 |
| Peduncles of equal length | 5 |
| 5 Penis with 10 or 13 hooks | <i>C. prionti</i> (MacCallum, 1917) Price, 1943 |
| Penis with eight hooks | 6 |
| 6 Body proper oval, anterior end obtuse, peduncles comparatively short and robust | <i>C. labracis</i> (Cervantaine, 1895) Price, 1943 |
| Body proper lanceolate, anterior end rather narrow, peduncles comparatively longer and slender | <i>C. elongata</i> (Goto, 1894) Price, 1943 |
| 7 Three anterior pairs of peduncles of equal size and relatively large, posterior pair relatively small, with smaller suckers | 8 |

	Peduncles progressively shorter in antero-posterior succession	9
8	Larger, with 25 testes and 12 cirrus hooks	<i>C. neomanis</i> (MacCallum, 1917) Price, 1943
	Smaller, with 56 to 65 testes and 13 cirrus hooks	<i>C. caulolatai</i> (Meserve, 1938) Price, 1943
9	Origin of the anterior-most peduncles contiguous	<i>C. pagelli</i> (Gallien, 1937) Price, 1943
	Origin of the anterior-most pair of peduncles separated by the width of the body, i.e., haptor not distinctly set off from body proper	10
10	Body ovate, peduncles of the posterior-most pair of haptoral suckers reach the haptoral disc separately, languette present, number of testes about 30	<i>C. chrysophryi</i> (Beneden and Hesse, 1863) Price, 1943
	Body elongate, posterior-most pair of haptoral suckers pedunculated but the two peduncles unite posteriorly into a single median stem, joining the haptor, languette absent, number of testes more than 30	<i>C. multatesticula</i> , n sp

The family *Microcotylidae* Taschenberg, 1879 is characterised by the possession of a variable number of haptoral suckers, the framework of the sucker consisting of seven pieces of which three are paired and one median. The family contains only one sub-family, *Protomicrocotylinae* Johnston and Tiegs, 1922 which contains only a single genus, *Protomicrocotyle* Johnston and Tiegs, 1922. Probably the genus *Microcotyle* van Beneden and Hesse, 1863, *Axme* Abildgård, 1794, *Bicytlophora* Price, 1936 and *Centracolpa* Meserve, 1938 which at present are not assigned to any sub-family will have to be grouped in a new sub-family *Microcotylinae*, n sub-fam. The genus, *Protomicrocotyle* is characterised by the possession of four suckers in one row on the posterior end of the body, a dumbbell-shaped haptor, numerous (150–200) testes and the position of the ovary in the posterior portion of the body. The form *Bilateracotyle chirocentrosus*, n.g. et n.sp. evidently belongs to a new genus of this sub-family because, it has three suckers in two rows on the posterior end of the body an oval haptor, only 20–28 testes, unarmed, long and tubular cirrus and more numerous spines of the genital atrium. The following is the genus diagnosis:—

Bilateracotyle, n.g.

Genus Diagnosis.—Sub-family. *Protomicrocotylinae* Johnston and Tiegs, 1922, with sub-family characters; body elongate, with two elliptical anterior

suckers. Intestinal crura not uniting posteriorly, with lateral ramifying branches specially on the outer side, posterior haptor disc oval in shape, bearing three pairs of hooks, two rows each of three typical microcotylid retractile, pedunculated suckers with variations in the orientation of the chitinous framework present at the posterior end of the body proper; genital atrium with 24-38 long spines, ovary situated in the middle of the body; vitellaria extending from the level of the intestinal bifurcation upto the suckers, testes 20-28, vesicula seminalis long and with well-developed prostatic gland cells Egg single, spindle-shaped with polar filaments parasitic on the gills of marine fishes

Type Species *B. chirocentrosus*, n sp

A new family *Gastrocotylidae* is recently created by Price (1943) and is characterised by the possession of numerous haptoral suckers and in having a skeletal framework, the arrangement of which is figured by him (Price, 1943, Fig 1 D, p 45). A detailed paper by him on the subject is not yet published. I have no hesitation in including the genus *Pseudaxine* Parona and Perugia, 1890, in this family because of the resemblance of its skeletal framework with the type described and figured by Price. Probably the genera, *Gastrocotyle* van Beneden and Hesse, 1863, *Gotocotyle* Ishii, 1936 and *Thoracocotyle* MacCallum, 1913 will also be included in this family.

The form *Pseudaxine indicana*, n sp belongs to the genus *Pseudaxine* because its haptor is separated from the body by a notch, and because it has a single row of suckers on the margin of the haptor and because the haptor has a haptoral outgrowth "Proboscis" with two pairs of hooks.

The genus includes the following four species *P. trachuri* Parona and Perugia, 1890 (Type species), *P. katsuwensis* Ishii, 1936, *P. vagans* Ishii, 1936 and *P. mexicana* Meserve, 1938. *P. indicana*, n sp differs from the above in the general shape of the body, in the number of testes and suckers, in the number and shape of hooks in the genital atrium and in the position of the ovary and particularly in the structure of the framework of the haptoral sucker.

I also propose to include the three forms *Pricea multæ*, *P. minimæ* and *P. microcotylæ* n sp, in a new genus of the family *Gastrocotylidae*, which I have named after Dr. Emmett W. Price in recognition of his work in this group of trematodes. In fact the structure of the framework of the haptoral sucker is widely different from the type one described for this family by Price (1943) and differs from all known genera. If Price's criterion for basing the classification of these forms on the structure and arrangement of the cuticular pieces of the framework of the haptoral sucker is valid, probably a new

family will have to be created for these three forms. There are a large number of other differences as well. But since this family is in a process of revision by Price himself I content myself with creating the new genus without at present proposing a new family for it. The genus *Pricea*, n.g. has the following generic diagnosis —

Genus *Prices*, n.g.

Genus Diagnosis — Family *Gastrocotylidae* Price, 1943 Body elongate, with two elliptical oral suckers, intestinal cæca discontinuous, with ramifying lateral branches into the vitellaria specially on the outer sides, terminating before the beginning of the haptor, a pair of hooks present at one end of the haptor and one pair above it in the posterior portion of the body proper Testes post-ovarian 25–38, follicular, not extending beyond the ends of the intestinal crura Vitellaria follicular, extending from the level of genital pore anteriorly to the termination of the intestinal cæca posteriorly Vaginal opening situated at the point of bifurcation of œsophagus into intestinal limbs, having a pouch and a U-shaped cuticular hook Genital pouch situated on the œsophagus halfway the distance between the pharynx and œophageal end It has twelve cirrus hooks Ovary situated in the mid-region Haptoral suckers with a characteristic structure (Fig. 38), situated on both sides of the haptor in double row, the number varying from 70–122 (Figs 29, 37 and 43) They may be pedunculated Excretory pores two, lateral, situated in the region slightly below the brain Parasites of marine fishes

Type species *Pricea multæ*, n.sp

The three forms included in this genus differ from each other in the shape and size of the body and haptor, number of suckers, extent of vitellaria, number of testes and position and size of body hooks

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TREMATODES FROM INDIANM ARINE FISHES

Part IV. On Some Trematodes of the Family *Hemiuroidae*, Luhe, 1901,
with Description of Six New Forms

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I INTRODUCTION

THIS paper is one of the series of papers on the trematodes of the marine fishes of the Indian coast. Parts II and III of this series have already been published (Chauhan 1943). Part I dealing with some new ectoparasitic monogenetic trematodes is under publication. This paper describes six new trematodes of the family *Hemiuroidæ*.

The specimens were collected at Bombay in November-December 1939, and December-January 1940-41.

II DESCRIPTIONS

Order —*Digenea* (Van Beneden, 1858) Carus, 1863

Sub-order —*Prosostomata*, Odhner, 1905.

Super-family —*Hemiuroidæ*, Poche, 1925

Family —*Hemiuroidæ*, Lühe, 1901

A Sub-family —Hemiuirinæ, Lühe, 1901

Genus —Aphanurus, Looss, 1907

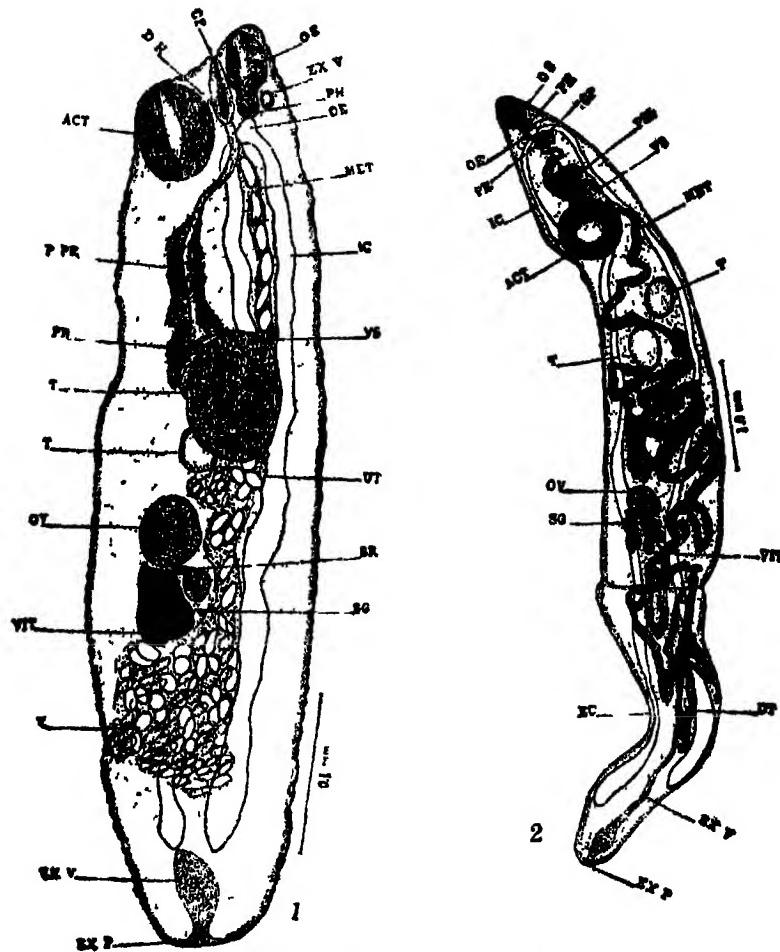
(1) *Aphanurus microorchis*, n.sp.

(Fig. 1)

Body elongately oval, anterior end pointed, posterior broad; very minute transparent forms measuring 0.58 mm in length and 0.144 mm in maximum width (in the region of vitellaria), skin beset with minute cuticular spines; oral sucker (*OS*) terminal, oval in shape, measuring 0.025 × 0.039 mm, Pharynx (*PH*) compact and muscular, elongate, measuring 0.013 × 0.019 mm, followed by a small oesophagus (*OE*) bifurcating posteriorly into two intestinal cæca (*IC*) extending a little short of the posterior end. Ventral sucker (*ACT*) large and oval measuring 0.063 × 0.05 mm. situated very close to the oral sucker. Testes (*T*) two, small, spherical, situated one behind the other immediately anterior to the middle of the body. The distance between the two testes is 0.018 mm and their diameter 0.025 mm. Vesicula seminalis (*VS*) very large, spherical, measuring 0.081 × 0.062 mm, situated in the region of testes. Pars prostatica (*P PR*) not highly developed, the duct is sinuous and opens into the ductus hermaphroditicus along with the metraterm (*MET*). Prostrate gland cells (*PR*) well developed. Ductus hermaphroditicus (*DH*) a short thick, conical sac measuring 0.013 × 0.041 mm, situated in between the two suckers and opening to the exterior on the ventral side near the oral sucker by a genital pore (*GP*). Ovary (*OV*), post testicular, spherical, measuring 0.039 × 0.044 mm and situated at a distance of 0.018 mm from the posterior testis. Receptaculum seminis (*SR*) and shell gland (*SG*) present. Vitellaria (*V IT*), characteristic of the genus, a single compact mass being made up of right and left vitellarium fused together, immediately behind the ovary, measuring 0.037 × 0.05 mm. Uterus (*UT*) partly inter-cæcal and mostly post ovarian in extent, the metraterm (*MET*) running anteriorly along the prostatic duct and opening into the ductus hermaphroditicus. Eggs (*E*) oval, large, average measurement, being 0.018 × 0.009 mm. Excretory pore (*EXP*) terminal, the vesicle (*EX V*) post-cæcal.

The sub-family Hemiuirinæ Luhe, 1901, contains four genera of which the genus *Aphanurus* is devoid of a tail and is further characterised by possessing a vitellarium mass made up of the right and left vitellarium masses fused together.

The genus *Aphanurus* was established by Looss in 1907, with *A. stossichi* (Monticelli, 1891) as the type species. The genus contains two other species *A. virgula* Looss, 1907 and *A. harenula* Yamaguti, 1938. The new

FIG 1 *Aphanurus microorchis*, n sp. Lateral view

ACT, Acetabulum, **DH**, Ductus hermaphroditicus, **E**, Egg, **EX P**, Excretory pore, **EX V**, Excretory vessel, **GP** Genital pore, **IC**, Intestinal cæcum, **MET**, Metraterm, **OE**, Oesophagus, **OS**, Oral sucker, **OV**, Ovary, **PH**, Pharynx, **PPR**, Pars prostatica, **PR**, Prostate gland cells, **SG**, Shell gland, **SР**, Receptaculum seminalis, **T**, Testis, **UT**, Uterus, **VIT**, Vitellaria, **VS**, Vesicula seminalis

FIG 2 *Lecithochirium polynemus* n.sp., Ventral view

EC, Ecsoma, **PSP**, Presomatic pit, other lettering as in previous figure

species described above differs from the other species of the genus in the relative position of the two suckers, size and extent of ductus hermaphroditicus, position of testes being not behind the vesicula seminalis and the relative size of vesicula seminalis, ovary and vitellaria. Eggs are larger, prostate gland cells do not extend all along the duct, and the skin is beset with minute cuticular spines.

Host.—*Mugil persia*

Location.—Alimentary canal

Locality—West coast of India, Bombay

B. Sub-family—Sternhuriæ Looss, 1907

Genus—*Lecithochirium*, Luhe, 1901

(2) *Lecithochirium polynemous*, n.sp.

(Fig. 2)

This tailed hemurid has an elongate body, tapering at both the ends; in living condition the worms are white, transparent forms with golden yellow eggs and dark brown vitellaria. It measures 7.53 mm in length with tail and 1.1 mm. in maximum width (in the region between the testes and ovary). Cuticle smooth. Presomatic pit (*P SP*) muscular, situated in between the genital pore and acetabulum on the ventral side usually doming over between the first and second portion of the tripartite *vesicula seminalis*. Tail (*EC*) retractile, about one-third the body length. Oral sucker (*OS*) subterminal, oval, measuring 0.23 × 0.26 mm, followed by a small compact muscular and round pharynx (*PH*) measuring 0.13 mm in diameter, œsophagus (*OE*) very small, the two sinuous limbs of the intestine (*IC*) extending into the tail upto nearly the posterior end. Ventral sucker (*ACT*) spherical, measuring 0.56 mm in diameter, situated in the middle of the first half of the body (soma). Testes (*T*), two, elongately oval, tandem-post-acetabular, situated in the middle of the body, the average measurements being 0.26 × 0.36 mm. *Vesicula seminalis* (*VS*) a curved broad sac tapering anteriorly and rounded posteriorly divided into three unequal portions; anterior portion continued into the small and round genital sinus through a long and thin S shaped duct. Genital pouch surrounded by glandular pars prostatica and opening to the exterior through a genital pore (*GP*), in the region immediately below the bifurcation of the intestine in the mid-ventral axis. *Vesicula seminalis* entirely pre-acetabular. Ovary (*OV*) spherical, situated behind the testes, in the last quarter of the body, at a distance of about 1.0 mm from the posterior testis and measuring 0.26 × 0.33 mm. Receptaculum seminis present, shell gland (*SG*) small. Vitellaria (*VIT*) situated immediately behind the ovary in two groups. The right mass has three small thick lobes on the outer side, the left four—their shape is much varied. Uterine coils (*UT*) extend into the tail and are more heavy in the tail and in between the region between the ovary and posterior testis, anteriorly the uterus runs on the right side of the ovary, takes a turn

in between the testes, again runs on the right side above the anterior testis and below the acetabulum, finally the metraterm (*MET*) opens into the genital sinus. Eggs oval, measuring 0.01×0.014 mm (average). Excretory vesicle (*EX V*) Y shaped with a dilatation in the posterior end; excretory pore (*EX P*) terminal.

The sub-family *Sterrhurinae* Looss, 1907 contains many genera of which *Lecithochirium* Luhe, 1901 is characterised by the possession of a presomatic pit. The genus *Lecithochirium* was created by Luhe in 1901 with *L. rufoviride* (Rud.) 1819 as the type species. Since then other species have been assigned to the genus, viz., *L. gravidum* Looss, 1907, *L. dillanei* Nicoll, 1918, *L. caudiporum* (Rud.), *L. synodi* Manter, 1931, *L. exodicum* Macfarlane, 1936, *L. japonicum* Yamaguti, 1938, *L. microstomum* Chandler, 1935, *L. magnapossum* and *L. murænae* Manter, 1940.

The species described above differs from all the known species of the genus in the posterior extent of the intestinal cæca and uterus into the ecosoma, in the disposition of the uterine coils, relative position of the gonads, the nature of male end-genital ducts and the extent of vesicula seminalis.

Host - *Polynemus indicus*

Location — Intestine

Locality — West coast of India, Bombay

(3) *Lecithochirium scutus*, n.sp.

(Fig. 3)

Body elongate with the anterior end above the acetabulum tapering and the region of soma behind the acetabulum (*ACT*) with parallel sides; tail (*EC*) short, cuticle smooth, length (with the tail completely retracted) 5.4 mm, maximum width 1.0 mm., anterior end with a dorsal nippleshaped, small and flat preoral lip (*OL*). Presomatic pit (*PSP*) with well developed muscle fibres and transverse oval opening, situated slightly below the middle of the distance between the genital opening and acetabulum. Oral sucker (*OS*) subterminal, small and oval measuring 0.13×0.21 mm. Pharynx (*PH*) small, compact, round and muscular, measuring 0.11 mm. in diameter. Oesophagus very small; the two cornua of the intestine do not extend into the ecosoma. Testes (*T*), two, spherical, tandem, situated just behind the acetabulum in the anterior third of the body, anterior testis measures 0.31×0.42 mm.; the posterior is slightly bigger and measures 0.34×0.47 mm. Vesicula seminalis (*VS*) a very massive, thick and sinuous tripartite sac extending beyond the anterior end of the ventral sucker; the

anterior end long and thin opening into the genital sinus through a ductus hermaphroditicus (*DH*) and surrounded by a pars prostatica (*P PR*) consisting of gland cells, arranged all round the duct in an oval mass; the ductus hermaphroditicus is a small, hollow, muscular, sinus opening to the exterior through a genital pore (*GP*), situated on the left side of the oesophagus and is probably surrounded by a few muscle fibres. Ovary (*OV*) spherical, situated behind the testes, slightly behind the middle of the body, at a distance of 0.81 mm from the posterior testis, measuring 0.31×0.26 mm Receptaculum seminis (*SR*) small, Vitellaria (*VIT*) situated immediately behind the ovary in two groups, one group consisting of three thick closed finger-shaped lobes and the other usually 4 lobes on their outer sides Uterine coils (*UT*) heavy, below the vitellaria and on their right side end in between the posterior testis and ovary, anteriorly the uterus passes by the left side of the posterior testis and acetabulum over the presomatic pit, the terminal end, metraterm (*MET*) opens into the genital sinus Eggs elliptical, the average measurements being 0.015×0.01 mm Excretory vessel (*EX V*) Y shaped.

This species differs from all the known species of the genus in the peculiar shape of the oral lip, the relative size and ratio of the two suckers, the size and positions of testes and ovary and the nature of genital sinus, position of pars prostatica and the nature and extent of the massive and sinuous vesicula seminalis

Host—*Arius fulcarius*

Location.—Alimentary canal

Locality—West coast of India, Bombay

Some specimens obtained from *Mugil persica* resemble closely the species *L. polynemus*, obtained from the same locality and described above, but differ from it in that the uterine coils do not extend into the ecosoma and lie most heavily in between the ovary and the acetabulum; the vesicula seminalis is shorter and more club-shaped than that of *L. polynemus*, the presomatic pit is more highly developed and is longer in size and the preoral lip is club-shaped Vitelline follicles are three on the right and only two on the left side The tail is also shorter I regard these differences as important variations but I do not venture at this stage to propose that they represent a new species. I shall examine more specimens, if possible

C Sub-family —Dinurinae Looss, 1907

(a) Genus — *Lecithocladium* Luhe, 1901

(4) *Lecithocladium annulatum*, n.sp.

(Fig. 4)

Body cylindrical with elongated tapering ends, skin of soma beset with strong saw-like cuticular rings with lateral dentitions, more pronounced

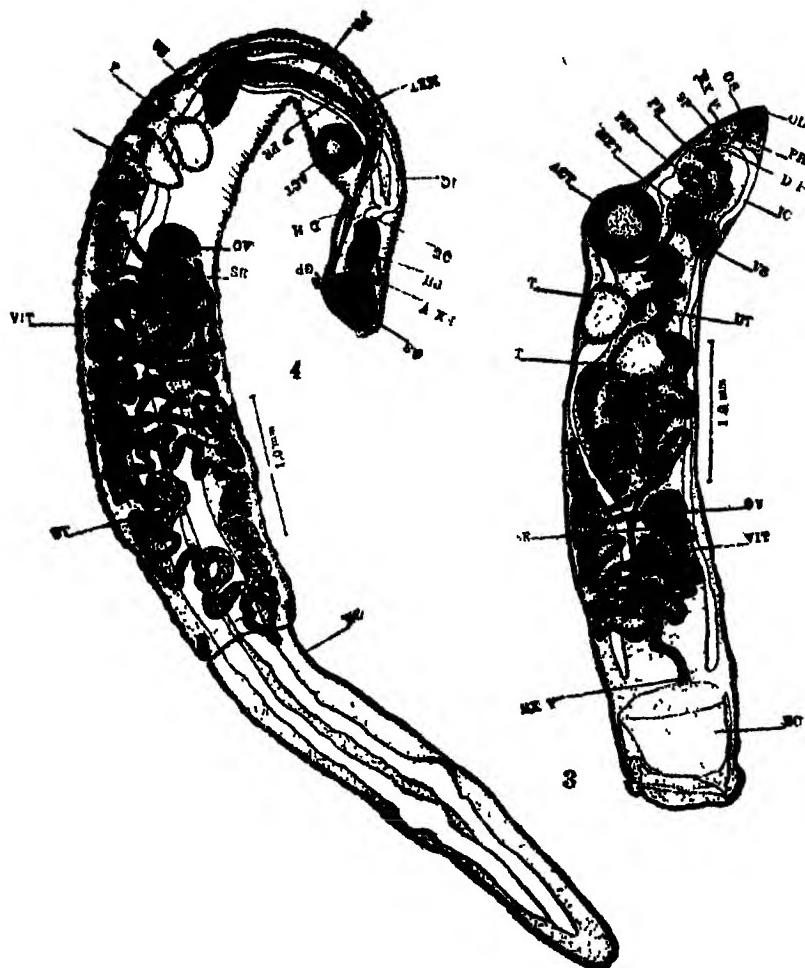


Fig. 3 *Lecithochirium acutus*, n. sp., Lateral view
OL. Preoral lip, other lettering as in previous figures.

Fig 4 *Lecithocladium annulatum*, n.sp., Lateral view
Lettering as in previous figures

in the anterior region, length 9.87 mm, Fig 4 maximum width below the vitellaria 1.1 mm, tail (EC) measures 3.61 mm, oral sucker (OS) terminal, cup-shaped with a prominent dorsal lip and measuring 0.425×0.325 mm. Pharynx (PH) elongate, cylindrical, with the shape characteristic of the genus, measuring 0.4×0.175 mm. Oesophagus (OE) short and oval, the two intestinal limbs extend into the tail upto nearly the posterior end. Ventral sucker (ACT) spherical situated at about one-sixth body length, measuring 0.35 mm in diameter. Testes (T) two, oval and separate lying one behind the other just posterior to the vesicula seminalis, their average measurements being 0.4×0.2 mm. Vesicula seminalis (VS) a pear-shaped organ pointed posteriorly and measuring 0.25×0.425 mm, situated at a distance of about one third the body length and with muscular walls. Pars prostatica (PPR) long and tubular running upto a little anterior to the acetabulum and surrounded by poorly developed prostate gland cells (PR). Ductus hermaphroditicus (DH) a short straight tube, measuring about 0.7 mm and extending only upto oesophagus, it opens to the exterior through a genital pore (GP) situated on the antero-ventral margin of the oral sucker, where the sucker has developed a groove. Ovary (OV) kidney-shaped situated at a distance of 0.25 mm from the posterior testis and measuring 0.35×0.25 mm. Receptaculum seminis (SR) present, a spherical body measuring about 0.15 mm in diameter. Vitellaria (VIT) consist of seven long filiform tubes entirely post-ovarian in two groups of four on the left and three on the right. Uterine coils (UT) mostly placed in the body behind the ovary. Uterus extends anteriorly on the right side of the testes as metraterm (MET) to join the tubular pars prostatica to form the ductus hermaphroditicus. Eggs small, elliptical, measuring 0.012×0.0048 mm. The arms of the excretory vessel (EX V) observed only in the 'bed head'.

The genus *Lecithocladium* is distinguished from the other genera of the sub-family Dinurinae by the possession of a funnel-shaped oral sucker, long and cylindrical pharynx and an unpartitioned vesicula seminalis. The prostate gland cells are confined only to the posterior part of the duct.

This genus was created by Luhe in 1901 with *L. excisum* (Rudolphi 1819) Luhe, 1901 as the type species. Srivastava (1942) lists the following species under the genus *L. excisum* (Rud., 1819) Luhe 1901; *L. excisiforme* Cohn, 1903, *L. gulosum* Linton, 1910; *L. longucaudum* Shen Tseng, 1935, *L. psenopsis*, *L. magnacetabulum* and *L. pagrosomi* Yamaguti, 1934 and *L. johni* Yamaguti, 1938. He further adds two new species *L. harpodonitis* and *L. brevicaudum* Srivastava, 1937.

The new species *L. annulatum* resembles in its general appearance *L. harpodonitis* but differs from it in that its ductus hermaphroditicus is not

sinuous but is a very short straight tube, not extending even upto the acetabulum; acetabulum is situated more anteriorly and the pars prostatica is more elongate, vesicula seminalis is differently shaped, has muscular walls and is situated anterior to testes. Cuticle is prominently ringed; oesophagus and receptaculum seminis are not absent; the tail is comparatively longer, testes separate, and the number of vitelline tubes is only seven (eight in *L. harpedontis*).

Looss (1908) regards *L. excisiforme* Cohn, 1903 to be synonymous with *L. excisum* (Rud., 1819). Srivastava (1942) regards it a valid species. He does not include *L. cristatum* (Rud., 1819) in his list of valid species of the genus, but I am inclined to do so. I am also inclined to transfer the species *L. longicaudum* Shen Tseng, 1935, to the genus *Stomachicola* Yamaguti, 1934. I think the corrugated nature of the tail of *L. magnacetabulum* Yamaguti, 1934 is due to its tail being well extended.

Host.—*Stromateus cinereus*

Location.—Alimentary canal

Locality.—West coast of India, Bombay

(5) *Lecithocladium glandulum*, n.sp.

(Fig. 5)

Body short, elongate, spindle shaped with the anterior end broad and tapering and the posterior pointed, skin of the body (soma) beset with cuticular annulations more pronounced in the anterior region, length 3.62 mm., maximum width 0.51 mm in the region of vitellaria, ecsoma (EC) short and stumpy, measuring 1.12 mm. A humplike 'skin-spur' (NB) with radially arranged muscle fibres termed by Rudolphi (1819) as "Nacken-buckel" is present on the skin in the region of pharynx on the right side. Oral sucker (OS) subterminal, funnel-shaped with a dorsal broad and prominent oral lip (OL); the sucker measures 0.18 × 0.16 mm. It leads into an elongate cylindrical pharynx (PH) measuring 0.11 × 0.175 mm. Oesophagus (OE) short. The two intestinal limbs extend into the tail upto nearly the posterior end. Ventral sucker (ACT) spherical, situated slightly below one-third the body length measuring 0.2 × 0.225 mm. Testes (T) two, equal, spherical and separate, situated obliquely one behind the other in the posterior half of soma, their average diameter being 0.175 mm. Vesicula seminalis (VS) elongately oval with thick muscular walls post-acetabular and situated partly in the region right of the anterior testis measuring 0.25 × 0.11 mm. Pars prostatica (PPR) sinuous, long and tubular running anterior to the acetabulum, to open into the ductus hermaphroditicus, along

with the metraterm surrounded by well-developed prostate gland cells (*PR*) extending nearly upto the middle of the acetabulum. Ductus hermaphroditicus (*DH*) is a short, straight tube lying in a sinus sac, measuring about 0·325 mm and extending posteriorly slightly below the shoulders of the intestinal cæcum, anteriorly it runs closely by the side of the pharynx on its right side and opens to the exterior through a genital pore (*GP*) in the region of the passing of the oral sucker into the pharynx. Ovary (*OV*) dome-shaped situated just posterior to the posterior testis and separated from it only by a few uterine coils. Receptaculum seminis (*SR*) big, shell gland (*SG*) round. Vitellaria (*VIT*) consist of eight short and thick filiform tubes arranged into two groups of four each, on either side, situated immediately behind the ovary, slightly above or in the region of the meeting of the soma with the ecosoma. Uterine coils (*UT*) extend posteriorly into the tail, on the intestinal cæca upto about two third the ecosoma length, anteriorly the uterus runs on the right side of the ovary in between the ovary and the posterior testis and the two testes and finally the metraterm (*MET*) runs on the left side of the male efferent duct and opens into the ductus hermaphroditicus. Eggs (*E*) elliptical, their average measurements being $0\ 024 \times 0\ 01$ mm.

The species *L. glandulum* described resembles *L. pagrosomi* Yamaguti, 1934, in general appearance, out of all the known species of the genus. However it differs from the latter in that it has a very short ductus hermaphroditicus which is long, sinuous and originates at the level of the middle of the acetabulum in *L. pagrosomi*. The genital pore of the new species is situated near the anterior end of the pharynx and not on the ventral margin of the oral sucker, the cervical gland (Nacken-buckel) is distinct; ovary is differently shaped, intestinal cæca are not unequal, testes are smaller and not contiguous with one another and with the ovary and the eggs are larger, acetabulum is situated more posteriorly and the vitellaria are in groups of four each and not five and three, metraterm extends much anterior to the acetabulum and the tail is shorter.

Host — *Lutjanus johnii* and *Mugil speigleri*

Location — Intestine

Locality — West coast of India, Bombay

(6) *Lecithocladium carultum*, n.sp.

(Fig. 6)

Body truncated with the anterior end pointed and posterior broad. Cuticle weakly annulated, the annulations being more marked and close in the anterior region, length of the body 2·22 mm; maximum width

0.44 mm. in the region of soma posterior to vitellaria. Tail appendix (EC) short, Oral sucker (OS) subterminal, oval and measuring 0.19 mm. in diameter, pre-oral lip (OL) dorsal, flat and crescent-shaped, pharynx (PH) elongately oval measuring 0.11×0.175 mm., oesophagus (OE) very short; the two caeca of the intestine extend into the tail a little short of the

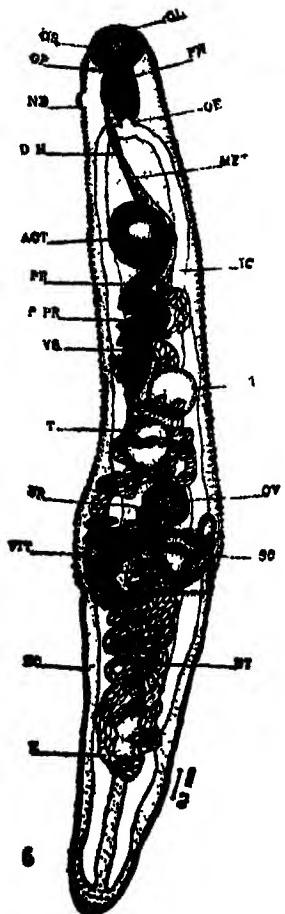
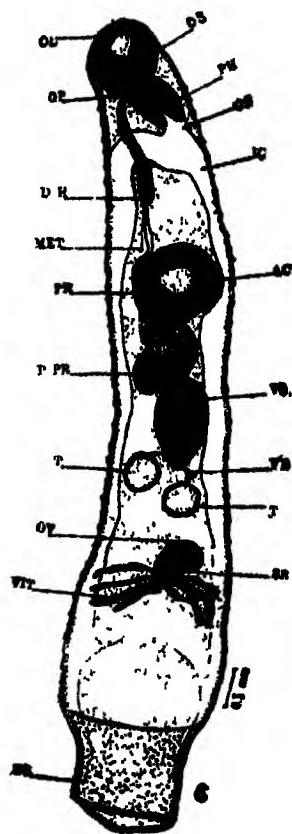


Fig 5 *Lecithocladium glandulum*, n.sp., Ventral view
NB, "Nacken Buckel", other lettering as in previous figures

Fig 6 *Lecithocladium carinatum*, n.sp., Ventral view
VE, Vasa efferentia, other lettering as in previous figures



posterior end. Ventral sucker (ACT) spherical, situated at one-third the soma length, measuring 0.21 mm. in diameter. Testes (T), two, small, spherical and separate, tandem lying posterior to the vesicula seminalis, their average diameter being 0.1 mm. The two vasa efferentia are seen

to pass anteriorly into the vesicula seminalis (*VS*) which is very big, pear-shaped, with highly muscular walls, measuring $0\ 26 \times 0\ 15$ mm and lying in the middle of the body. Pars prostatica (*PPR*) is sinuous, long and tubular surrounded by prostate gland cells (*PR*) only upto the anterior border of the acetabulum. Ductus hermaphroditicus (*DH*) is a comparatively long, broad and thick sac originating about midway between the ventral sucker and oesophagus running by the right side of the Pharynx and opening to the exterior by a genital pore (*GP*), situated on the posterior border of the oral sucker, at the right side of the pharynx. It measures $0\ 32$ mm in length. Ovary (*OV*) small, pear-shaped, measuring $0\ 11 \times 0\ 05$ mm and situated at a distance of $0\ 07$ mm from the posterior testis. Receptaculum seminis (*SR*) large and round, measuring $0\ 06$ mm in diameter. Vitellaria (*VIT*) thin, small and filiform tubes, eight in number, situated immediately behind the ovary in two groups of four each. Uterine coils were not seen but the metraterm (*MET*) is on the right side of the prostatic duct, four mature, long and elliptical eggs were seen in the ovary, their average measurements being $0\ 018 \times 0\ 006$ mm.

This species differs from all the known species of the genus in the relative size of the gonads, receptaculum seminis, vesicula seminalis, and vitelline tubes. The shapes of the vitelline tubes, ovary, ductus hermaphroditicus and pharynx are also distinctive. The relative position and ratio of the suckers, extent of prostatic gland cells and the size and structure of ductus hermaphroditicus are characteristic of the species. Eggs are comparatively very long.

Host—*Sciaena carulta* and *Harpodon nehereus*

Location—Alimentary canal

Locality—West coast of India, Bombay

(7) *Stomachicola muranesocis* Yamaguti, 1934

The genus *Stomachicola* was created by Yamaguti (1934) with *S. muranesocis* Yamaguti, 1934 as the type species. I have a large number of representatives of this parasite from the *Muraenesox talabonoides* from the Bombay coast. These forms show a great degree of variations. Both Yamaguti (1934) and recently Bhalerao (1943) have referred to most of them.

Shen Tseng (1935) described a new parasite *Lecithocladium longicaudum* from *Muraenesox cinereus* (Forskal) from China. I am inclined to transfer this species to the genus *Stomachicola*. Incidentally it may be pointed out that the hosts of both Bhalerao's forms and Shen's forms happen to be exactly the same.

III ACKNOWLEDGMENT

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EMBRYOLOGICAL AND CYTOLOGICAL STUDIES IN THE FAMILY ASCLEPIADACEÆ

2. An outline of Meiosis in *Dæmia extensa* Br., with special reference to the role of nucleolus

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[Communicated by Prof H Chaudhuri, D.Sc. (Lond), F.A.S.C.]

INTRODUCTION

In connection with the embryological studies of the local members of the family Asclepiadaceæ on which work has been in progress in this laboratory, the material of *Dæmia extensa* turned out to be peculiarly interesting for a detailed cytological investigation. It was, therefore, thought advisable to extend the scope of the problem and work out details of meiotic cytology as well. Particular care was devoted to the study of nuclear activities preceding spindle formation with the object of elucidating to some extent the significance and destiny of nucleolus, the role of which is even to-day very imperfectly understood. A number of interesting features have been observed and the present account is just an outline synopsis of the outstanding stages of meiosis with special reference to the origin, behaviour and function of the nucleolus in the process.

FIRST DIVISION

Before the advent of the prophase the cytoplasm of the resting microspore mother-cells was a nonvacuolate, finely granular mass while their nuclei showed a faintly-staining reticulum and a single prominent deep-staining nucleolus.

The first visible indications of the approaching prophase were seen in the nucleolus which showed budding or vacuolation though sometimes the two processes proceeded simultaneously (Fig 1). While these changes were in progress, the thready nature of the nuclear reticulum became more definite. The threads were dispersed throughout the nucleus without any definite orientation. They were single and not longitudinally divided anywhere. They could, further, be followed to a considerable length but their exact relationship to each other could not be clearly made out. The threads were evenly disposed in the nucleus and had faintly-stained chromomeres.

As a result of budding and vacuolation, the nucleolus became, gradually, split up into a number of fragments which were seen scattered amongst the threads (Fig 2) The author has very definite reasons to believe that the ultimate number of these nucleolar fragments was 12, which corresponds with the haploid number of chromosomes in the plant The synzetic contraction described by various investigators but now believed as an artefact, was not observed in any preparation The most interesting feature of the early prophase was, however, the eventual complete disappearance of the nucleolar fragments and the almost contemporaneous appearance of the deeply-stained chromomeres profusely studding the threads (Fig 3). This represented the ultimate phase of the leptotene stage In view of the above facts the nucleolus is obviously the sole storage organ for chromatin but the precise manner in which the chromatin flows to the leptotene threads yet remains to be properly determined

The leptotene stage was of a short duration and was soon followed by the zygotene and pachytene stages The homologous chromosomes came together in pairs and the paired threads of each bivalent coiled round one another Subsequently the threads underwent a considerable shortening and thickening and after the usual diplotene stage in which the chromosomes fall apart, the diakinesis was reached The chromosomes had, by this time, attained their almost maximum lengthwise contraction and had become much shorter and thicker Eventually, as generally happens in the late prophase, the thickened chromosomes moved to the periphery of the nucleus and arranged themselves on the inside of the nuclear membrane (Fig 4)

During prometaphase, the nuclear membrane was observed to become thinner and thinner while within it there appeared a zone of faintly-stained, indistinct fibrils which later developed into the spindle The origin of the spindle was thus intranuclear The diakinesis bivalents contracted still further and became associated with the developing spindle and as the metaphase stage was reached, the 12 bivalents gathered on the equator without undergoing any further condensation. The univalents were quadrangular in outline

The anaphase and telophase stages were passed over rather quickly, the former having been observed at its early split There is a very strong evidence in favour of the fact that during telophase, the 12 univalents at each pole of the spindle segregated into faintly-stained linin threads and the deeply-stained chromatin pieces, the latter underwent contraction and became spherical.

Before taking up the account of interphase, it may be mentioned that the heterotypic division was regular and reductional, the haploid number of chromosomes being 12. Further, neither the nucleolus nor even any of its fragments was seen in any of the above stages after leptotene.

INTERPHASE

There was a well-defined period of rest during the interphase. A careful study of the several preparations at this stage showed that the twelve contracted spherical chromatin pieces which had separated from the 12 univalents at each pole of the spindle, underwent gradual coalescence and eventually gave rise to a daughter-nucleolus. Originally 12 such pieces were seen enclosed by the nuclear membrane, most of them occupying the peripheral position (Fig. 5, left-hand daughter-nucleus). Later on a pair fusing together, the number went down to 11, one of them being evidently larger in size (Fig. 5, right-hand daughter-nucleus). In this way, the number decreased by gradual steps to 2 (Fig. 6) and eventually to 1 which was the daughter-nucleolus fully formed. At an early stage of the interkinesis, granules began to be deposited at the equatorial region and very soon there originated from them a cell-wall which separated the daughter-nuclei.

SECOND DIVISION

The homotypic division then followed and the grand-daughter nuclei whose nucleoli originated, presumably, like those of the interkinetic-nuclei became separated by walls parallel to the one already existing. Each microspore mother-cell thus gave rise to a linear tetrad.

CONCLUSION

The above account would clearly indicate that in connection with the meiotic cytology of *Dæmia extensa*, there are a number of points of special interest such as the intranuclear origin of the achromatic figure, a well-defined period of rest during interkinesis, deposition of the granules at the equatorial region leading to cytokinesis and a very significant rôle of the nucleolus. The structure of the nucleolus in the resting condition of the microspore mother-cell and its subsequent behaviour during the two meiotic divisions establish beyond doubt that it is a storehouse for chromatin and that there exists a specific relationship between the nucleolar material and the cyclic alterations of chromosomes. It is further inferred that there is a definite correspondence between the number of chromatin pieces of which the nucleolus is composed and the haploid number of chromosomes i.e. 12. It is not possible to give in this note, the details of evidence in this direction nor the discussion that the subject really deserves. In a recent review on

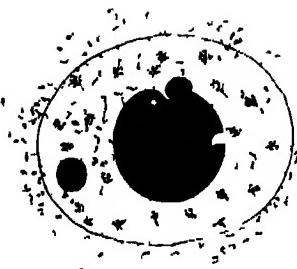


FIG. 1 ($\times 1333$)

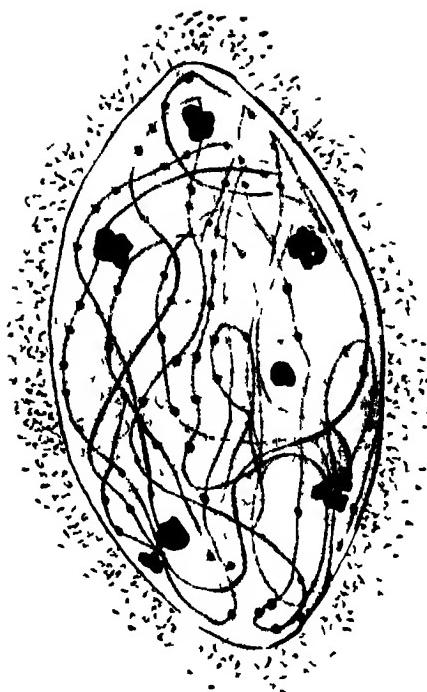


FIG. 2 ($\times 1333$)

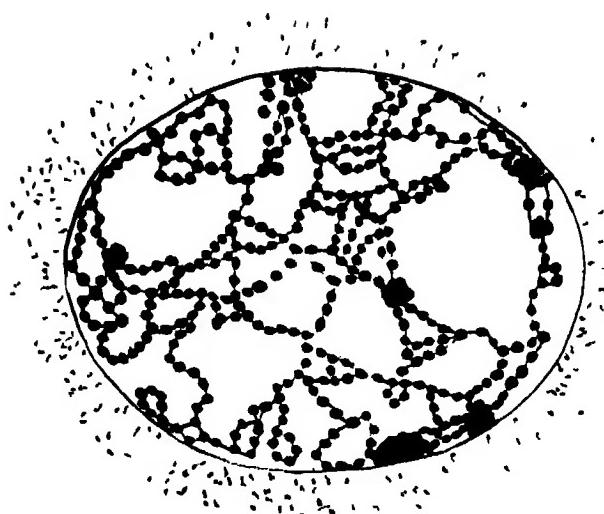


FIG. 3 ($\times 1333$)

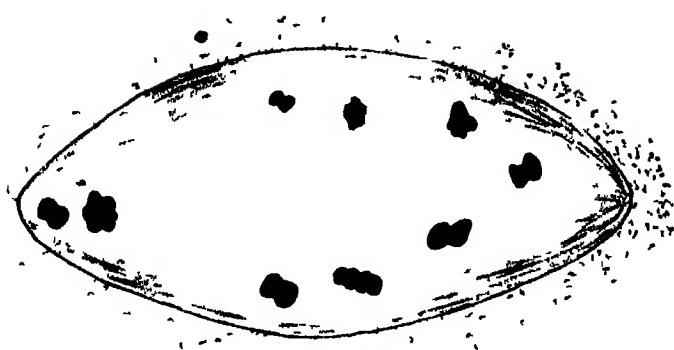


Fig. 4 ($\times 1333$)

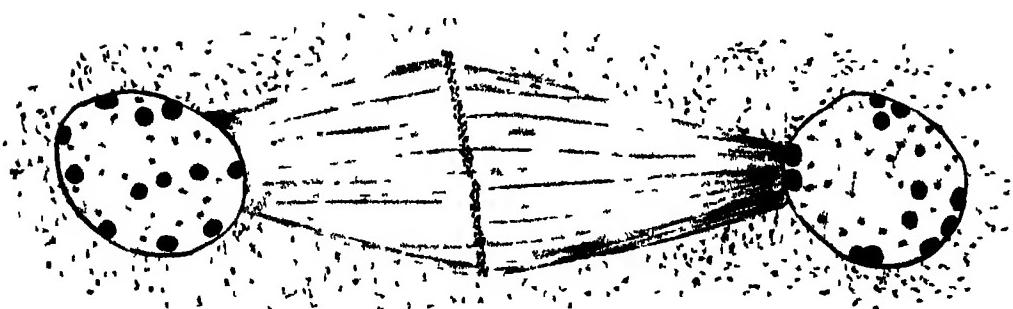


Fig. 5 ($\times 1333$)

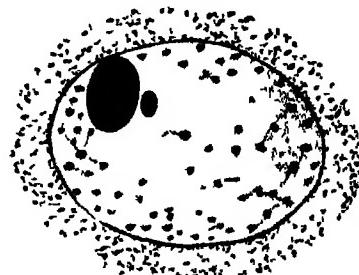


Fig. 6 ($\times 1333$)

nucleoli and the related nuclear structures, Gates⁴ has given an exhaustive account of the subject in all its aspects and has quoted an extensive literature at the end. Some of the most notable workers whose cytological investigations have thrown a great deal of light on the origin and history of the nucleolus are Bhatia,¹ van Camp,² Gates,³ Heitz,⁶ Latter,⁸ Ludford,⁷ McClintock,⁸ Nandi,⁹ Nawashin¹⁰ and Zirkle¹¹. Of particular interest in connection with the present investigation are the findings of van Camp² and Nandi.⁹ The former was of opinion that the nucleoli originated from the chromosomes at telophase in the form of small globules which later by fusion formed one large nucleolus. According to the latter the nucleolar material got liberated during the heterotypic telophase as small globules which, under the influence of the nucleolar body present in one chromosome of the haploid complement were organised into a definite nucleolus that remained associated with one particular chromosome. In the case of *Damia extensa* of which the account has been given above, each haploid chromosome gave rise to a deeply-stained chromatin piece during the heterotypic telophase so that ultimately there originated 12 such pieces and these coalesced together to form one nucleolus during interphase. A detailed account of all the facts would be published later.

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EMBRYOLOGICAL AND CYTOLOGICAL STUDIES IN THE FAMILY ASCLEPIADACEÆ

3. The development and arrangement of Microspores in *Hemidesmus indicus* Br.

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Received May 24 1944

[Communicated by Prof H Chaudhuri, D.Sc (Lond.), F.A.S.C.]

INTRODUCTION

FROM amongst the various investigators on the embryology of Asclepiadaceæ, Frye¹ and Gager² have pointed out a large number of peculiarities in *Asclepias*. In regard to the microsporogenesis, they described two microsporangia in a stamen, direct conversion of the sporogenous cells into spore mother-cells, several layered tapetum around a microsporangium and finally linear tetrads of microspores. Work on the local Asclepiadaceæ was started some years ago to determine whether there were really any features which could be said as characterising the family. During the course of that work, some interesting facts have come to light on the microsporogenesis of *Hemidesmus indicus* Br., with special reference to the direction of the homotypic spindles and the subsequent arrangement of the tetrads.

ORIGIN AND DEVELOPMENT OF MICROSPORANGIA

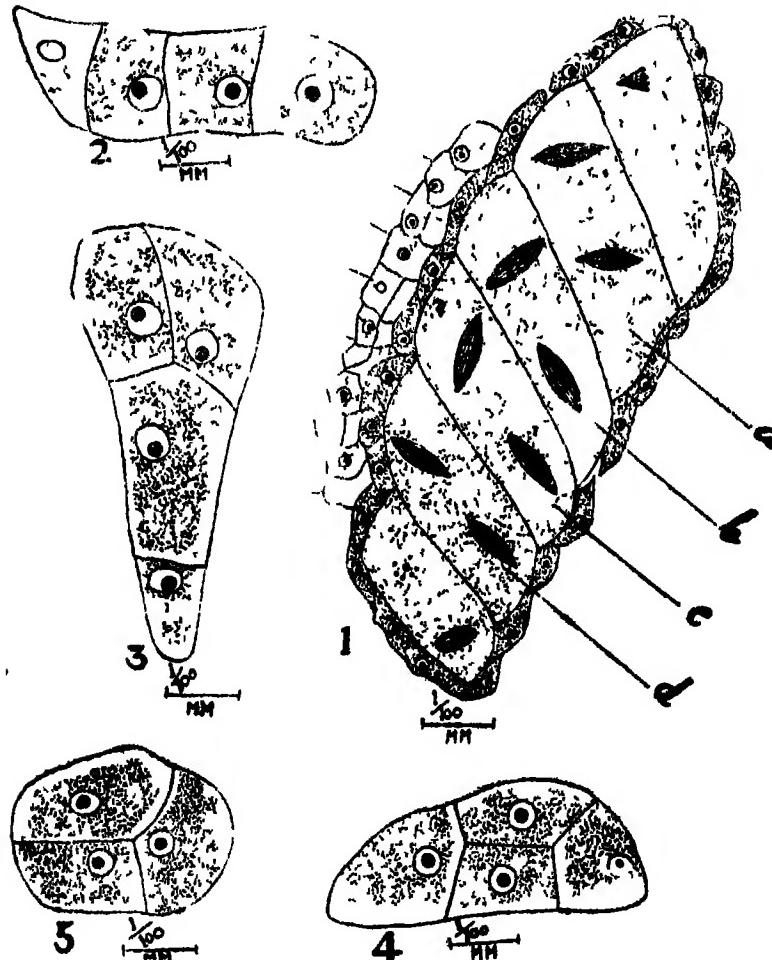
A flower in *Hemidesmus indicus* produced, around its central axis, five syngenesious stamens each of which gave rise to four distinct sporangia. The latter is rather an unusual number in the family since all the earlier workers have mentioned only two microsporangia. It may, however, be mentioned that one of us³ drew attention to several such cases on a previous occasion.

The primary archesporium consisted of a row of 6 or 7 cells which divided in the usual manner by periclinal walls to form the primary parietal cells towards the outside and the primary sporogenous cells on the side. The cells of the primary parietal layer divided by periclinal walls and gave rise to three concentric layers. The sporogenous cells underwent considerable elongation in the radial direction and became converted into spore mother-cells. Towards the outside of the sporogenous tissue, the tapetum was organised from the innermost parietal layer. A microsporangium when fully developed was reniform in appearance with a distinct groove towards one

side and was surrounded by a one-layered tapetum of uninucleate cells. The microspore mother-cells were generally narrower towards the groove side and were comparatively broader on the other. As observed by the senior author⁴ in *Daemia extensa*, their cytoplasm was a non-vacuolate finely granular mass while their nucleus showed a faintly-staining reticulum and deep-staining nucleolar matter.

ARRANGEMENT OF TETRADS

The most interesting features that struck the authors in their study of the microsporogenesis are related to the direction of the spindles and the



consequential arrangement of spores in their tetrads. The heterotypic spindles were situated in the linear direction of the microspore mother-cells.

and generally occupied a somewhat central position. The homotypic spindles, however, displayed a considerable diversity in their disposition (Fig 1). The spindle which was on the narrower side of the microspore mother-cells was situated generally lengthwise (Fig 1, b, c, d) while the one that lay on the broader side showed a great variation, in some cases it was at right angles to the length of the microspore mother-cells (Fig 1, b), in others it was slightly tilted from a strict right-angled position (Fig 1 c) and ultimately in some, it was in the linear direction and was in line with the spindle which was towards the narrower side (Fig 1, d). There were also cases where both the spindles were placed in the breadthwise direction of the microspore mother-cells, i.e. parallel to one another (Fig 1 a). The last condition was observed in the microspore mother-cells which were more or less equally broad at both the ends.

The walls separating the microspore nuclei were laid down after both the divisions were complete though in one case an ephemeral wall was seen after the heterotypic division. In accordance with the varying directions of the homotypic spindles and the consequential position of the daughter nuclei in different microspore mother-cells, there resulted different types of tetrads such as linear (Fig 2), T-shaped (Fig 3), tetrahedral (Fig 4), iso-bilateral (Fig 5) and even some other intermediate ones. The microspore formation was thus by the simultaneous division and led to several types of tetrads. It would not be out of place to mention that Wille¹ described varying arrangements of microspores in species of *Juncus* and *Orchis masculata*; and in *Typha* Schaffner² not only found the tetrads indiscriminately tetrahedral or bilateral but frequently the four spores were in a row. Maheshwari³ has described tetrahedral and bilateral arrangements in *Boerhaavia diffusa* and Rao⁴ has come across these two types in *Gynandropsis pentaphylla*. In Asclepiadaceæ the tetrads are so far reported to be either linear^{5,6,7,8} or tetrahedral⁹. The present communication which records a variety of tetrads in one and the same plant is thus significant and noteworthy.

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EMBRYOLOGICAL AND CYTOLOGICAL STUDIES IN THE FAMILY ASCLEPIADACEÆ

4. On the development of Embryo-sac and Endosperm in *Daemia extensa* Br.

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Received June 26, 1944

[Communicated by Prof H Chaudhuri, D SC (Lond), F A SC]

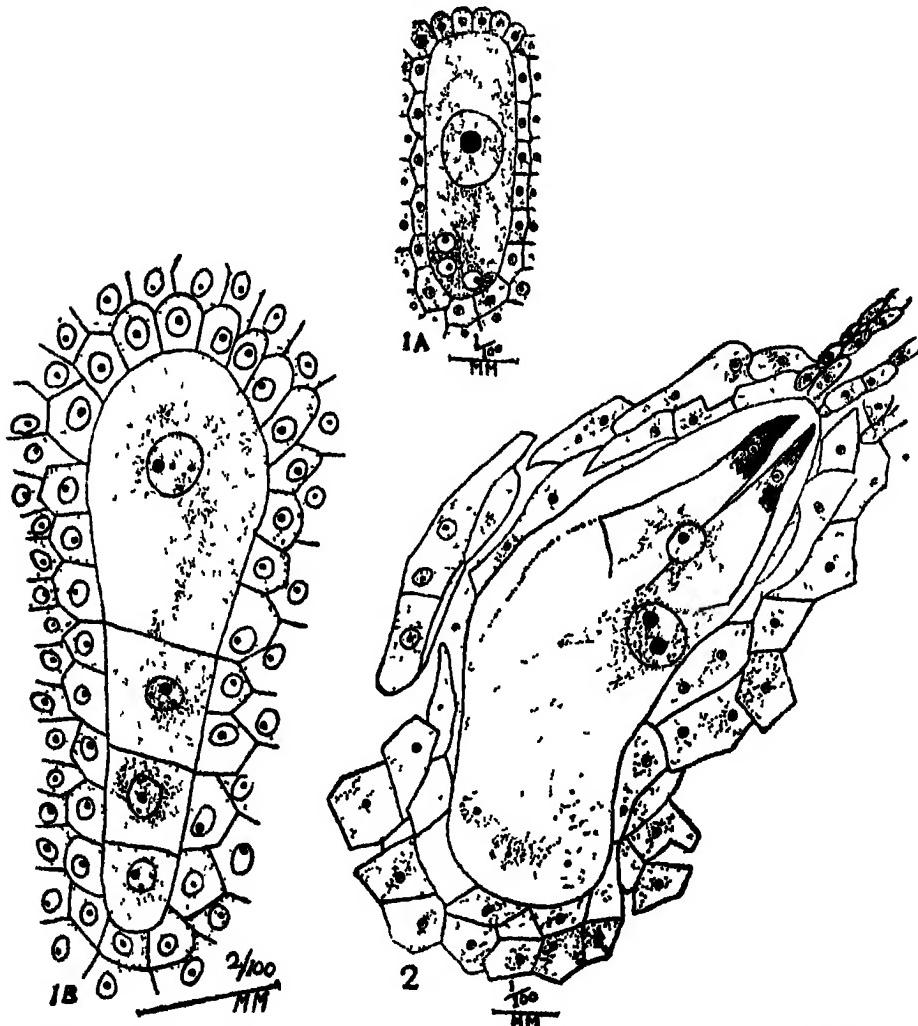
INTRODUCTION

A SEARCH through the available literature on megasporogenesis and embryogeny of Asclepiadaceæ has shown that Frye³ described in *Asclepias*, several-celled archesporium, a compact antipodal tissue and finally 16 to 32 free endosperm nuclei before the egg showed any sign of segmentation. Similarly Sabet⁶ by his study of *Calotropis procera* observed several features of interest such as small antipodals, additional and abnormal embryo-sacs and the fertilised egg resting until the endosperm had become cellular and had almost filled the sac. The latest contribution on the embryology of Asclepiadaceæ is by Pardi⁵ who investigated the development of megaspores and embryo-sac in seven species of five genera and described linear, T-shaped and inverted T-shaped tetrads, 8-nucleate normal embryo-sacs, small antipodals and distinctly pointed and beak-like synergids. For some time past, the writer also has been studying this part of the life-history in *Daemia extensa* and has come across a number of features which deserve special mention.

MEGASPOROGENESIS AND ORGANISATION OF THE EMBRYO-SAC

Almost simultaneously with the formation of microspore tetrads, hemispherical protuberances begin to bulge out from the placentas and in course of time each constitutes the nucellus of the nascent ovule. At an angle of the naked nucellus, a hypodermal cell recognised by its increasing size and larger nucleus forms the archesporium which directly functions as the megaspore mother-cell. Its nucleus after two successive divisions gives rise to four megaspores which are generally arranged in the normal linear fashion (Fig 1 B) but occasionally inverted T-shaped tetrads are seen. An L-shaped tetrad which appeared to be somewhat intermediate between the linear type and the inverted T-shaped one was also seen, this had another peculiarity, viz., the absence of walls between the respective spores. Whether

the walls were not laid down at all or that they were formed and disappeared later could not be determined with respect to this case. It is, however, of interest to note in this connection that in *Carex acuta*, Juel⁴ has reported the absence of wall formation during the development of microspores from the microspore mother-cell. Similarly in *Eichhornia crassipes* inves-



tigated by Smith,⁷ the four megasporangia of the linear tetrad are not separated by walls. In all cases of different types of tetrads, it is the outermost megasporangium that functions while the other three begin to disintegrate (Figs. 1 A and 1 B). This is an interesting feature, for in the large majority of

Angiosperms, it is the innermost megasporangium that grows further while the outer three degenerate. In Asclepiadaceæ also, usually the lowest megasporangium forms the embryo-sac as mentioned by Sabet⁶ and Pardi⁶ though Frye³ and Francini^{1,2} have reported the occasional functioning of other megasporangia as well. It appears that about the time, the lower megasporangia are undergoing degeneration, a single massive integument develops and surrounds the nucellus almost over-topping it so that the ovule apparently looks naked. The functional megasporangium enlarges considerably and as in *Damia tomentosa*⁸ elongates, becomes slightly curved and narrows down at one of its extremities. Ultimately it becomes deeply seated in the ovule by the activity of cells lying above.

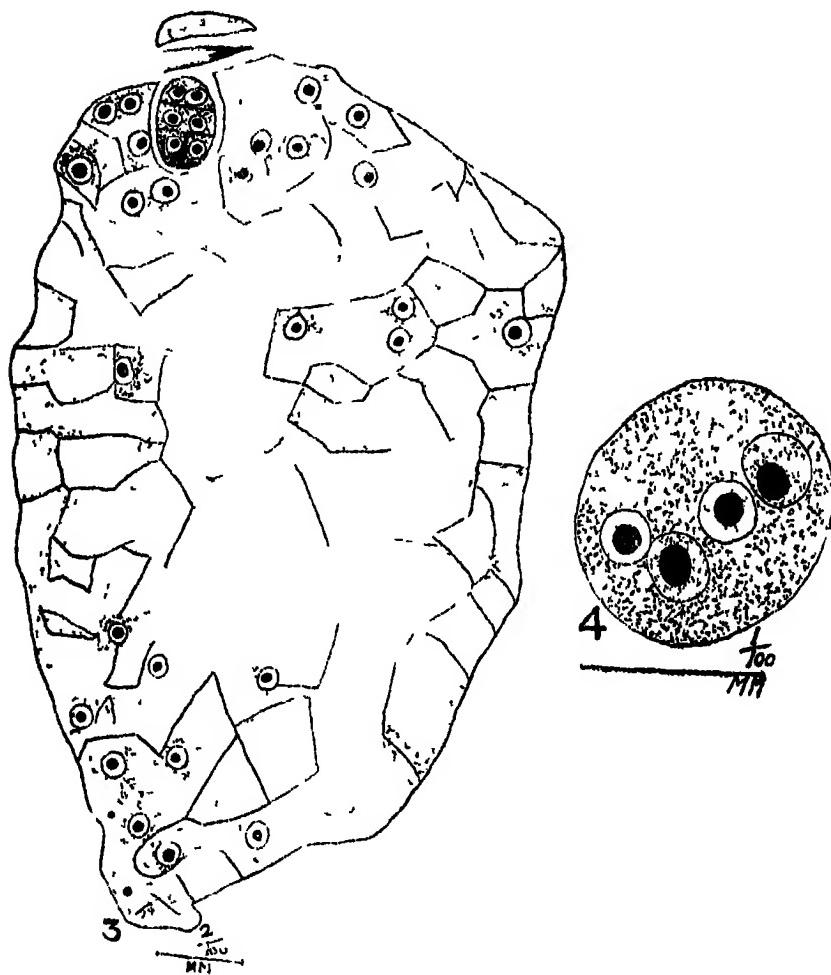
The history of the gametophyte from the megasporangium to the completion of the egg-apparatus is of the normal type. The antipodal cells are small, ephemeral and probably take no part in the activities of the embryo-sac. The polar nuclei fuse before the synergids are fully developed though sometimes their fusion is postponed to just before fertilisation. In conformity with the pointed micropylar extremity of the sac, the mature synergids develop beak-like extensions which, further, show delicate longitudinal striations (Fig. 2). At this stage a long and narrow micropyle can be seen on one side and the disintegrating antipodal cells on the other. With respect to the above characters of the embryo-sac, it may be mentioned that Pardi⁶ described in several species of Asclepiadaceæ the pointed beak-like synergids and Sabet⁶ observed the short life of the antipodal cells in a few genera of the same family.

FERTILISATION AND DEVELOPMENT OF THE ENDOSPERM

The pollen tube and the discharge of its contents are observed in a number of preparations and it is noticed that in the fertilised ovules there are generally no synergids. These evidently become disorganised during fertilisation though in one case their remains were seen as late as the production of proembryo.

The primary endosperm nucleus is always the first to divide and as the binucleate condition is reached, the ovule increases in its size until it acquires a tapering elongated form. In the earlier stages, endosperm develops by free nuclear division and it is at the 8-nucleate stage that walls first appear. Sabet⁶ who observed walls at the 16-nucleate stage, emphasised the fact that early cell-wall formation in endosperm is a common feature in Asclepiadaceæ. The endosperm now begins to develop with a remarkable rapidity and for some time there is no sign of the division of the egg. A rudimentary proembryo was seen for the first time when the endosperm

had already formed an extensive tissue (Fig 3) In this case, the endosperm had a large number of nuclei near the proembryo and was almost encircling it.



PROFMBRYO

The fertilised egg rests for some time and, as indicated above, does not start its development until a certain amount of endosperm has been formed. It is probably at the 32-nucleate stage that the first segmentation of the egg takes place. The first division could not be seen but Fig 3 leaves no doubt that it is transverse and that the second one is parallel to it, so that a proembryo of three cells in a row is formed. The same figure

indicates that the end cell divides by a longitudinal wall and that the upper two cells also divide longitudinally though in this particular case the dividing walls are not yet laid down

An abnormal proembryo with four nuclei (Fig. 4) was observed in one case. It did not, as yet, show any wall formation but from the position of the nuclei, it is obvious that the early divisions were irregular.

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ON A NEW FLAGELLATE, *PENTATRICHOMONAS ALLENI* N.SP., FROM THE INTESTINE OF THE HIMALAYAN CROW, *CORVUS LEVAILLENTI INTERMEDIUS* ADAMS

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THE first record of *Pentatrichomonas* in birds is given by Allen (1936) who found this organism in certain cases of enterohepatitis or 'blackhead' of turkeys, chickens, and guinea fowls. In view of Dobell's (1934) statement that the number of flagella in *Trichomonas* varied from three to five (e.g., in *T. hominis*) and therefore is not a specific character, Allen refrained from giving a specific name to the flagellate, although she stated that "four different strains of *Pentatrichomonas* isolated from poultry reveal that this is an organism having five anterior flagella, this number remaining constant except during mitosis, when it is doubled."

In this article we propose to describe a *Pentatrichomonas* which we encountered in the alimentary tract of three crows shot at Mukteswar in August 1942. One of the crows was very heavily infested with this flagellate, while the other two were infested with a species of *Isospora* in addition. On careful examination, we found that this organism was morphologically quite distinct from the one described by Allen. We therefore propose to designate it as *Pentatrichomonas allenii* n sp., the specific name being given in honour of Ena A Allen. No lesions associated with this organism were seen in any of the crows. Attempts to infect young chicks with this flagellate gave negative results.

TECHNIQUE

Allen's modification of Donaldson's iodine-eosin method was used for counting the number of flagella in fresh preparations of the intestinal contents. Permanent preparations of the flagellates were made by fixing the wet films in Schaudinn's fluid and subsequently staining them with Heidenhain's iron-hæmatoxylin and Dobell's (1942) ammonium molybdate-hæmatoxylin. Preparations with Leishman's stain were also made by Chatterjee's method (Ray, 1944). We have so far been unsuccessful to grow this flagellate in culture.

MORPHOLOGY

The live flagellate were very active and presented a spindle-shaped appearance with a slight depression along one of the margins towards the anterior end (Fig 1) The cytostome, which was represented by a narrow slit, was situated in this depression In the fixed and stained preparations, the anterior end presented a rounded appearance, probably due to flattening which occurred during fixation (Figs 4-7) In preparations stained with Leishman's stain, the region of the body anterior to the nucleus stained pink while the rest stained blue The cytoplasmic structure of the region, stained blue, appeared to be vacuolated, while the pink-stained area appeared to be of a very finely granular or hyaline nature (Figs 4 and 5)

A small number of granules, stained purple with Leishman's stain, were often found to be scattered in the cytoplasm posterior to the nucleus The body measured 7μ to 11.2μ in length and 2.8μ to 4.2μ in breadth

AXOSTYLE

The axostyle was represented by an axial fibre,—not a hollow tubular structure—which stained pink or purple with Leishman's stain and deep black and blue with Heidenhain's and Dobell's haematoxylin respectively (Figs 4-7) In some cases, it was found to project beyond the body posteriorly It originated from a blepharoplast which gave rise to the rhizoplast The poultry *Pentatrichomonas*, as described by Allen, has a rod-like axostyle

BLEPHAROPLAST AND FLAGELLA

At first there appeared to be a single blepharoplast giving rise to five free flagella, the undulating membrane and the axostyle, but in a few well spread out specimen, it was clearly seen that the different structures just referred to, arose from five different blepharoplasts in the following manner. the first blepharoplast gave rise to the first pair of flagella, the second one to the second pair of flagella, the third one to the bordering filament of the undulating membrane and the basal fibre, the fourth one to the axostyle and the rhizoplast, and the fifth one to the fifth flagellum (Fig 3) In wet fixed preparations, a rhizoplast originating from the fourth blepharoplast was seen to be connected with the nuclear membrane The *Pentatrichomonas* from the poultry is stated to have two blepharoplasts It was not possible to locate any rhizoplast connecting the blepharoplasts with each other The first pair of flagella was invariably found to be shorter in length than the second pair In this respect it differed from the poultry *Pentatrichomonas* which possesses four flagella of approximately equal length These two pairs of flagella lashed about in harmony with each other, the first pair rising up first to be followed by the second pair Frequently the flagella of each group were intertwined and were thus mistaken for only two or three flagella The

fifth flagellum had an independent movement and in stained smears appeared to be directed invariably towards the posterior end. In length the fifth flagellum was equal or slightly longer than the second pair of flagella.

UNDULATING MEMBRANE

The undulating membrane consisted of two to four irregular undulations. Like the poultry flagellate, basal fibre attached to the base of the undulating membrane, was also present here. The bordering flagellum became free posteriorly.

NUCLEUS

The nucleus was invariably situated in the centre of the body or slightly above it. In this character it differed from the poultry *Pentatrichomonas*, the nucleus of which was situated near the anterior end. The chromatin was distributed on the nuclear membrane and there was a central karyosome (Fig. 3). Although about 500 specimens were examined, no dividing form could be seen.

DISCUSSION

Allen (1936) described, from the poultry, an organism which shows a constant number of five free flagella. The present organism also possesses a similar character. Chatterjee, Ray and Mitra (1927) isolated from the intestine of an Indian jackal a flagellate, *Pentatrichomonas canis aurei* having also a constant number of five flagella. Derrieu and Raynaud (1914) were first to detect the organism with five anterior flagella in the stools of a patient suffering from chronic dysentery. They referred it to the genus *Hexamastix* Alexeieff (1912) and called it *H. ardindeitei*. Chalmers and Pekkola (1916) mistook it for a *Hexamita* which they called *Octomitus hominis*. Mesnil (1915) changed it to *Pentatrichomonas*. Chatterjee (1915) almost simultaneously described the same five-flagellated variety in Calcutta. In 1917 he recorded 32 cases of chronic dysentery from Bengal, in all of which *Pentatrichomonas* was present. Kofoid and Swezy (1923) reported three cases of infection with *Pentatrichomonas* in man in U.S.A. Das Gupta (1926) made cultural examination of the stools from 23 patients from Calcutta and the only organism encountered in all of them was *Pentatrichomonas*. Knowles (1928) found *Pentatrichomonas* to be the only form prevalent in Calcutta. *Pentatrichomonas* by usage has occupied a subgeneric rank in protozoological literature, although Dobell (Dobell and O'Connor, 1921) remarks: "I regard these so-called subgenera as varieties." Dobell (1934) also considers that the number of flagella in trichomonads is not a specific characteristic since he found the number of flagella in *T. hominis* to vary from three to five in pure strains. The systematic position of the *Pentatrichomonas*, therefore, is very uncertain at present. Nevertheless,

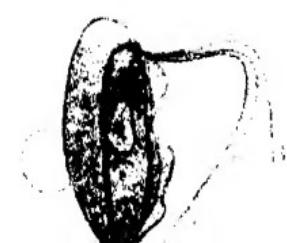
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2

3

4

5



A New Flagellate, P. alleni, from the Intestines of Himalayan Crow 189

the weight of available evidence indicates that there exists an organism with five free flagella and that four of these occur together in a group and the fifth is independent and that this number is a constant feature. In considering its relationship with other trichomonads, it would be unfair to ignore this special characteristic of *Pentatrichomonas*. We therefore, propose that *Pentatrichomonas* should be raised from the subgeneric to the full generic rank and not considered as a mere variety.

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EXPLANATION OF PLATE

Pentatrichomonas alleni n.sp

All figures were drawn with the aid of camera lucida either from fresh or stained preparations of the gut contents of the crow. Fig. 3 is diagrammatic and is highly magnified while the magnification of the rest is $\times 2,500$.

Figs 1 & 2 From a fresh preparation.

Figs. 3. Diagrammatic representation Highly magnified

Figs. 4 & 5 Stained by Chatterjee's method

Fig. 6 Fixed in Schaudinn's fluid and stained by Heidenham's iron-hematoxylin.

Fig. 7 Fixed as above and stained by Dobell's ammonium molybdate-hematoxylin.

SOME OBSERVATIONS ON THE SOIL FAUNA OF COTTON FIELDS AT LYALLPUR

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INTRODUCTORY

It has been estimated that about 95 per cent. of the insects live in the soil during some stage of their life-cycle. It is, therefore, evident that a number of our serious crop pests, must have a subterranean existence during some stage. The Desert Locust, the Rice Grasshopper and most other Orthoptera lay their eggs in the soil, so does *Monophlebus (Coccidae)*. A number of our pests spend their larval stage underground and it has been recognised that in certain cases more serious injury may be caused by the subterranean stages than by the forms above ground. For instance, at times the larvae of *Aulocophora abdominalis (Chrysomelidae)* are very harmful because they bore into the main roots and stems and cut off the food supply and very often cause well developed melon vines bearing excellent crop, to wither away within a few days. Again, the activities of the apodous grubs of *Myllocerus maculosus (Curculionidae)*, which live and feed underground, cause far greater damage than their leaf eating parents. Trehan (1928) has estimated that one grub of *M. maculosus* may destroy nine germinating cotton seedlings before it is full fed. The fruit flies, cut-worms, the notorious *Katra (Amsacta morei) Trachæ* sp. and many other pests, pass their pupal stage in the soil.

The root-aphis and the white ants spend their entire life underground and often cause serious damage.

It is, therefore, evident that a study of the insect fauna of the soil is of as great an importance for an applied entomologist, as a study of the fauna above ground. Unfortunately very little attention has been given to this aspect of agricultural entomology in India.

The present investigation was taken in hand in 1927 with the object of making a survey of the soil fauna of cotton fields, at Lyallpur (Punjab). In all, ten different plots were kept under observation—five during the cotton season when these were under cotton crop and five during the offseason after the cotton crop had been removed.

I had hopes of extending the observations but it is regretted that it has not been possible on account of work of greater importance, to pursue this investigation further. The present paper is being published to stimulate interest in this important aspect of agricultural entomology and to place on record the data obtained although very meagre.

I gratefully acknowledge the assistance of the Imperial Institute of Entomology, London, in identifying some of the material, and the Agricultural Chemist to Government, Punjab, for supplying the data regarding moisture and organic matter in the samples. I am deeply indebted to K B M. Afzal Husain under whose guidance I carried out these investigations.

TECHNIQUE

Soil samples were obtained by digging out a block of soil 9" x 9" x 9" in three separate layers, each 3" deep. In order to obtain a representative record, samples were taken from different parts of each field.

Isolating organisms from the soil is a tedious affair. In the automatic apparatus designed by Berlese (1905) and improved by Tullgren (1917) the directive influences of heat, light and humidity were utilised. Tragardh (1933) found this method very useful in the case of Collembola but worthless for the purpose of collecting earthworms or small thin-skinned Acarina. Buckle (1921) spread out the soil sample to dry for three days, and then passed the soil through sieve. By this process a number of insects are sure to die and shrink and it is difficult to isolate or identify such material. Morris (1922) spread out the soil sample on a brown paper and examined small quantities at a time, later (1923) he adopted the method of washing samples through a set of sieves of different meshes. Davidson and Swan (1933) adopted a similar method, and placed the samples directly in water and picked out the floating insects. Recently a new apparatus has been introduced by Ladell (1936) in which the principle utilized is flotation by a dense liquid and the author describes certain advantages over other apparatus.

I however, obtained very satisfactory results by placing soil samples in bags of fine muslin cloth and washing away the soil under a tap or by suspending a number of the bags in a stream of running water—small irrigation channel. This did not involve any special apparatus and the samples were washed thoroughly much quicker and many samples could be treated at a time. After the soil had been washed away the residue was transferred to a glass dish containing clear water. The macro-organisms whether sunk or floating could be easily fished out by means of a pipette or a brush and the immature stages were reared in the laboratory as far as possible.

ECOLOGICAL DESCRIPTION OF THE PLOTS

1 Off-season plots

Plot A—Insectory Area of the Punjab Agricultural College, Lyallpur 150×37.5 ft., fallow, after the previous cotton crop, which had followed sugarcane Rotation, therefore was sugarcane, cotton, fallow. The soil was typical loam. Adjacent to it towards the East was a field under sugarcane and towards the West a field under leguminous crop. There was a wire fence towards the North, beyond which was a small water channel, and a wire fence on the South separated the plot from the road. The water channel influenced the moisture of the soil which decreased towards the South. The main slope was towards South-East. The area was exposed to wind and sun.

The surface vegetation was moderate and consisted of —

- | | |
|---------------------------------|---------------------------------|
| 1 <i>Chenopodium album</i> , | 2 <i>Convolvulus arvensis</i> , |
| 3 <i>Euphorbia prostata</i> , | 4 <i>Heliotropium supinum</i> , |
| 5 <i>Carthamus oxyacantha</i> , | 6 <i>Cynodon dactylon</i> |

Plot B—Students' Farm, the Punjab Agricultural College, Lyallpur. The rotation followed was wheat, cotton fallow. On its North was a plot lying fallow, wheat plots bordered it on the South and East and cotton fields on the West. The soil was loam but comparatively drier than in Plot A and was exposed to wind and sun.

The surface vegetation was quite good and consisted of

- | | |
|---------------------------------|--------------------------------|
| 1 <i>Sorghum halepense</i> , | 2 <i>Cyperus tuberosus</i> , |
| 3. <i>Cynodon dactylon</i> , | 4 <i>Chenopodium album</i> , |
| 5 <i>Cajanus indicus</i> , | 6 <i>Tribulus terrestris</i> , |
| 7 <i>Convolvulus arvensis</i> ; | 8 <i>Heliotropium supinum</i> |
| 9. <i>Carthamus oxyacantha</i> | |

Plot C—Students' Farm. It had practically the same conditions as plot B described above.

Plot D—Chak 279 at Lyallpur The plot had practically no surface vegetation The soil was clay loam, very hard and dry The rotation was wheat, cotton, fallow

Plot E—Chak 279, at Lyallpur Soil loamy the plot was under lucern which had followed cotton Thus it had a luxuriant growth of surface vegetation Both on the North and South of it tall trees formed wind breaks There was a cotton field on the West and a kiln on the East A small water channel was running on all its three sides, except on the West.

The surface vegetation was quite good and included —

- | | | | |
|----|---------------------------------|---|-----------------------------------|
| 1 | <i>Euphorbia hypercifolia</i> , | 2 | <i>Polygonum aviculare</i> , |
| 3 | <i>Conyza stricta</i> , | 4 | <i>Sorghum halepense</i> , |
| 5 | <i>Cassia</i> sp | 6 | <i>Euphorbia dracunculoides</i> ; |
| 7 | <i>Portulaca oleracea</i> , | 8 | <i>Medicago sativa</i> , |
| 9. | <i>Cynodon dactylon</i> | | |

II Plots under cotton

The fields selected were quite close to those described above

SOIL CENSUS

In all 55 samples were examined from the fields from where the cotton crop had been removed in January, and 38 from the fields where the crop was standing Samples were generally taken at about 8 hours in the morning The results of these observations are given in Tables I and III below

TABLE I

*Population at different depths in fields fallow after cotton
Observations from January to May, 1927*

Locality	Plots	Population at different depths			Total	Percentage of population at different depths			Remarks
		0"-3"	3"-6"	6"-9"		0"-3"	3"-6"	6"-9"	
Insectory Students' Farm	A	51	47	77	175	29.1	26.8	44.1	The Punjab Agricultural College Estate Lyallpur Private land, Lyallpur
	B	47	26	27	100	47.0	26.0	27.0	
	C	99	67	74	240	41.2	27.9	30.8	
	D	21	16	17	56	41.0	28.5	30.3	
	E	61	49	25	135	45.1	36.2	18.5	
		Average			40.7	29.1	30.1		

Data regarding moisture, organic matter and the surface vegetation in the plots under observation were also collected. The results obtained are

Some Observations on the Soil Fauna of Cotton Fields at Lyallpur 195

tabulated below whereas fluctuations in numbers at different depths and their proportions in different groups, as determined from the above figures, are represented in Figs 1 and 2

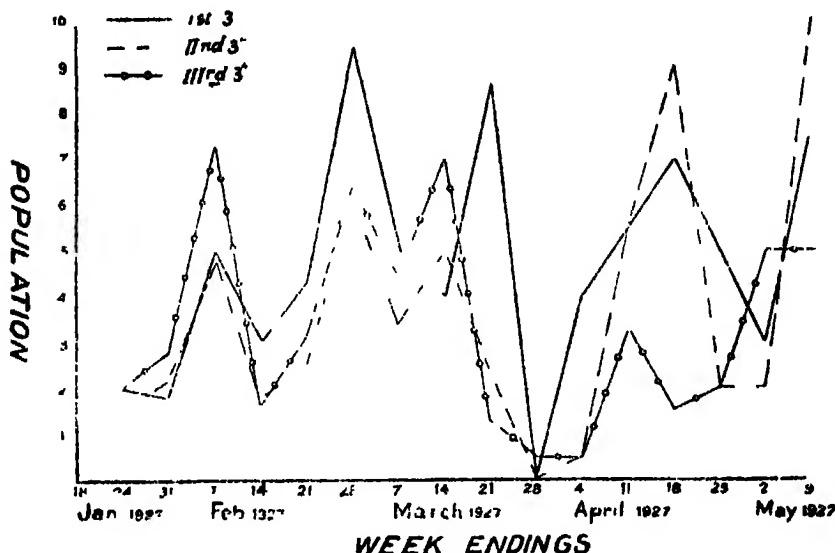


FIG. 1 Fluctuations in the population of fauna in plots fallow after cotton

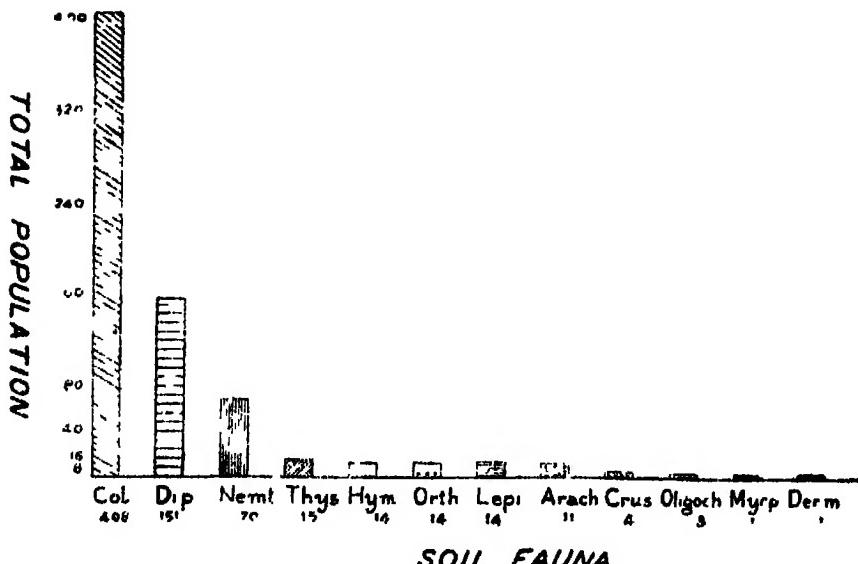


FIG. 2 Population of individual groups from the data collected during slack season

TABLE II
Moisture, organic matter, surface vegetation and faunal population

Locality	Plots and layers	Moisture percentage	Organic matter	Surface vegetation	Percentage of soil organisms	Remarks
Insectory	A I	12.02	1.13	Moderate	29.1	I Layer 1st 0"-3"
	A II	12.70	1.10		26.8	II Layer 2nd 3"-6"
	A III	13.40	2.07		44.1	III Layer 3rd 6"-9"
Students Farm	B I	3.43	1.31	Good	47.0	
	B II	5.34	1.66		26.0	
	B III	6.39	0.92		27.0	
Chak 279	D I	2.95	2.25	Practically nil	41.0	
	D II	5.15	1.86		28.5	
	D III	6.03	1.45		30.3	
Chak 279	E I	9.50	1.29	Very good	45.1	
	E II	9.75	1.67		36.2	
	E III	0.52	1.33		18.5	

It will be observed that the largest number of soil organisms was found in layer III in plot A and layer I in plots B, D and E (Table II). It appears likely that in the absence of surface vegetation the organic matter in the soil may also influence the distribution of population. The top 3" layer is, on the whole, richest in insect population.

The data collected during the cotton season are summarised below --

TABLE III
Population at different depths during the cotton season June-December

Locality	Plot No	Population at different depths			Total	Percentage of population at different depths			Remarks
		0"-3"	3"-6"	6"-9"		0"-3"	3"-6"	6"-9"	
Insectory Students' Farm	I	9	5	3	17	52.9	29.4	17.7	The Punjab Agricultural College Estate, Lyallpur Private land, Lyallpur
	II	31	42	22	95	32.6	44.2	23.2	
	III	46	19	8	73	63.0	26.0	11.0	
	IV	118	50	20	188	62.7	27.1	10.2	
	V	56	50	26	132	42.4	37.8	19.8	
Average					50.7	32.9	16.4		

FAUNAL ACTIVITIES

From the above data (Table III) it appears that during the cotton season the average population varied from 50.7 per cent, in the top 3" to 16.4 per

cent. in the 3rd layer Obviously therefore, the population decreased considerably with the depth of the soil During the slack season, however, (Table I) the average percentages of population in different layers varied from 40.7 in the top layer to 30.1 in the 3rd layer Under the cotton crop probably more favourable conditions are met with in the upper strata

Ploughing appears to have a distinctly destructive effect on the soil fauna in general The statement is supported by the fact that, in the plots under observation the number of *Myloocerus maculosus* grubs fell by about 75% after ploughing Besides direct injury, and attack of insectivorous birds, desiccation of soil proves fatal to the organisms

Draught and hardness of the soil which are factors responsible for reducing the number of soil organisms, are well compensated by the presence of surface vegetation or the organic matter in the soil, while, abundance of moisture in the absence of organic matter may have a detrimental effect

According to Cameron (1916) "difference in fauna of two areas possessing different soil types and vegetation covering is due to environmental conditions" Buckle (1921), however, suggests that most of the species occur irrespective of any soil type and majority of them adopted for hibernation or general feeding are not restricted to particular conditions The ecological conditions should be restricted to vast areas rather than to small ones Accordingly, therefore, distribution of fauna is more stable on grass land than on arable land as food is always available

Morris (1920) maintained that factors influencing the distribution of insects at various depths in the soil are chiefly the occurrence of food, aeration and moisture and it has been stated that they seldom penetrate below 6" even when the soil is covered over by snow Mostly they remain in the upper 2" soil.

It appears that food and moisture have a direct relation with the faunal distribution Surface vegetation plays an important part in determining the number and variety of soil organisms, firstly because of the supply of food, and secondly because the vegetation checks rapid evaporation and thus keeps the soil adequately moist

M'Atee (1907) calculated on an acre of forest near Washington, 1216, 880 animals belonging to the Annelida, Insecta, Gastropoda and Arachnida Cameron (1916) working on Clovers meadow estimated 835,560 insects per acre. On the basis of the data presented in Tables I and III, 1,006,371 and 1,033,250 organisms per acre are estimated with an average of 816,785 insects per acre. These estimates approach those of the above mentioned workers,

VERTICAL DISTRIBUTION OF THE FAUNA

Organisms occurred at all depths up to 12". Of the total number of individuals collected during this investigation 45.6 per cent were found in the uppermost 3" layer, 30.9 per cent in the 2nd layer and 23.5 per cent in the lowest 3" layer. These observations, however, are contrary to the results of Morris (1920), but are in conformity with his observations in 1927.

The distribution of the various groups in relation to the respective layers was as follows —

Nematoda I-III, Oligochæta III, Crustacea I-III,
 Arachnida I-III, Myriopoda II, Orthoptera I-III,
 Coleoptera I-III, Lepidoptera I, III,
 Thysanura I-III, Hymenoptera I-III,
 Rhynchota I, III, Diptera I-III;
 Mollusca was represented by empty shells only

RELATIVE ABUNDANCE OF DIFFERENT GROUPS

Of all the groups, the Insecta were the most numerous. Within the class the Coleoptera and Diptera were most numerous both in the number of individuals and species. Orthoptera, Lepidoptera and Thysanura were the next in importance and were followed by Hymenoptera, Rhynchota and Dermaptera which were poorly represented. Colonies of black and white ants, however, were not taken into consideration, and of the Apterygota only *Tvhsanura* was sorted out. Of the remaining groups, the Nematoda was the best represented.

The population of individual groups calculated on one acre basis is as follows —

Nematoda	131,510	Orthoptera	32,034
Oligochæta	2,529	Hemiptera	4,215
Arachnida	10,263	Lepidoptera	27,819
Crustacea	5,058	Coleoptera	504,120
Myriopoda	843	Hymenoptera	13,468
Thysanura	18,546	Diptera	257,118
		and unidentified eggs, etc	5,901

Of the pests of cotton the grubs and pupæ of *M. maculosus* were present and so were the white ants. Practically all the immature stages and even the newly emerged adults of *M. maculosus* were found in the 1st layer from April onward, but during winter, only the grubs could be recovered from below 3" (Fig. 3).

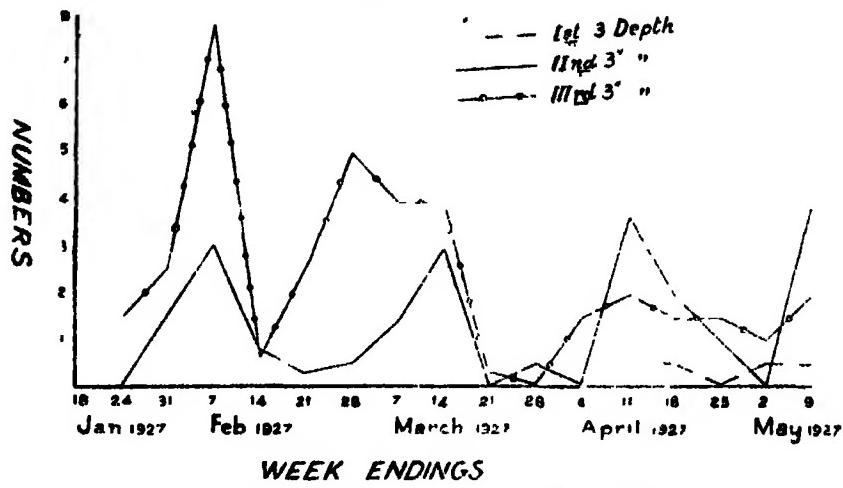


FIG 3 Hibernating grubs of *Myllocerus maculosus* at different depths

DATA

The following is the list of insects and other organisms showing their distribution during different months of the year and the depth at which they occurred.—

	Months off-season	Months cotton season	Total number of organisms	1 st layer in which found	Largest number in one sample	Layer in which found
1 INSECTA						
Coleoptera						
1 <i>Carabidae</i>	2-4	8-12	59	I, II	16	I
(i) <i>Platymetopus flavilabris</i> F	2		1	I		
(ii) <i>Tachys paerilopterus</i> Bates	2		4	I, II		
(iii) <i>Tachys babauiti</i> Andr	3		1	I		
(iv) <i>Tetragonolerus arcuatus</i> Dej		11	1	I		
(v) <i>Bembidion loricatum</i> Andr	2		1	I		
(vi) <i>Dromius adozus</i> Andr	1		1	II		
(vii) <i>Tachys tetraspilus</i> Solsky		12	1	I		
2 <i>Staphylinidae</i>	2-3	10-12	31	I-III	4	I
(i) <i>Platystethus cornutus</i> Gr	1-3	11, 12	14	I-III	5	
(ii) <i>Oleochara nitida</i> Gr (These were observed as parasitic on dipterous pupæ)		12	3	I, III		
(iii) <i>Oxytelus n sp</i>	2		2	II		
(iv) <i>Tachyporus</i> sp	3		1	II		
(v) <i>Pederus fucipes</i> Curt		12	4	II		
(vi) <i>Tachyporus nigromaculatus</i> Cam Var		12	1	I		
(vii) <i>Philonthus minutus</i> Boh		12	1	I		
(viii) <i>Philonthus anelensis</i> Boh		12	1	I		
3 <i>Pselaphidae</i>	2		1	I		
4 <i>Trogositidae</i>	4	6-8	6	I, II		

	Months off-season	Months cotton season	Total number of organisms	3 rd layer in which found	Largest number in one sample	Layer in which found
5 <i>Nitidulidae</i>		3	12	4	1	
(i) <i>Carpophilus</i> sp						
(ii) <i>Carpophilus dimidiatus</i> F						
6 <i>Coccinellidae</i>	1-2	8, 11	5	I, II		
(i) <i>Scymnus</i> sp	1, 2		2	I		
(ii) <i>Brumus</i> sp		11	1	I		
7 <i>Copridae</i>	1, 2, 4	11	7	I, III	3	II
(i) <i>Onditicellus pallipes</i> F						
(ii) <i>Onthophagus centricornis</i> F						
8 <i>Hydrophilidae</i>		12	1	I		
9 <i>Tenebrionidae</i>	3-5	11, 12	10	I-III	6	I
(i) <i>Mesomorphus striolatus</i> Fairm	3		1	I		
(ii) <i>Gonocaphalum vagum</i> Stern	5		6	I		
(iii) <i>Rhytinota impolita</i> Fairm						
(iv) <i>Oxycaria</i> n sp						
(v) <i>Gonocephalum</i> n sp						
10 <i>Mordellidae</i>	4	12	2	I		
(i) <i>Formicarius</i> sp	4		1	I		
11 <i>Chrysomelidae</i>	2-3	10, 12	11	I-III	2	II
12 <i>Lathrididae</i>	2		3	II, III		
(i) <i>Corticaria parnithorax</i> Champ	2		2	II		
13 <i>Elatiidae</i>		11	1	II		
(i) <i>Drasterius suteatulus</i> cand						
14 <i>Anthicidae</i>	4		1	III		
(i) <i>Anthicus communimacula</i> Fairm						
(ii) <i>Anthicus crinitus</i> Luf Var <i>Longipennis</i> Dcrbr						
15 <i>Pasvalidae</i>	2-4		5	I, III		
16 <i>Macrolonthidae</i>	2, 4	8-11	34	I, III	2	I
17 <i>Aphodiidae</i>	1-5	6-12	71	I, III	8	I-II
(i) <i>Rhysenus orientalis</i> Mots					7	I-II
(ii) <i>Aphodius lewisi</i> Watesh						
(iii) <i>Aphodius orientalis</i> Har						
(iv) <i>Aphodius</i> sp						
(v) <i>Psammohus incitus</i> Wlk						
(vi) <i>Aphodius granarius</i> L						
18 <i>Curculionidae</i>	1-5	6-12	277	I-III	15	III
(i) <i>Myllocerus maculosus</i> Desb						
(ii) <i>Echinocamus</i> sp ign						
(iii) <i>Atheracuta</i> sp (abraded)						
19 Unidentified specimens	1-5	7, 8, 10 12	41	I-III	5	I
Diptera						
1 <i>Dolichopodidae</i>	1, 2	11	21	I-III	2	I-III
2 <i>Chironomidae</i>	2-4	8-12	120	I-III	20	I
3 <i>Bibionidae</i>		10, 11	4	I, II	2	I, II
4 <i>Asilidae</i>	2		1	II		
5 <i>Dixidae</i>	11		1	II		
6 <i>Tachinidae</i>	4	12	5	I, III	2	I
7 <i>Sarcophagidae</i>	2		7	I, II	5	I
8 <i>Anthomyidae</i>	2, 3		6	I, III	3	I
(i) <i>Atherigona indica</i> Mall						
9 <i>Ornatidae</i>	11		1	III		
(i) <i>Chrysomyza demandata</i> F						
10 Unidentified specimens	1-5	6-12	113	I-III	15	I

Some Observations on the Soil Fauna of Cotton Fields at Lyallpur 201

	Months off season	Months cotton season	Total number of organisms	3" layer in which found	Largest number in one sample	Layer in which found
Lepidoptera	1-5	7, 9 10-12	32	I-III	4	I
1 <i>Cosmopterygidae</i> (i) <i>Anatrachyatis simplex</i>	1-4	10-12	24	I-III	4	
2 <i>Noctuidae</i>	3		3	I	2	
3 <i>Pyralidae</i>	4, 5	9	4	I, III	2	I
4 Unidentified	7		1	I		
Hymenoptera	2-4	11	14	I-III	3	I
1 <i>Apidae</i> (i) <i>Nomiaoides curvilineata</i> Cam	2		1	II		
2 <i>Braconidae</i>	2		1	II		
3 <i>Stephanidae</i>	11		1	II		
4 <i>Formicidae</i>	3-4		2	I, III	1	
5 Unidentified specimens	1-4	11	11	I-III	3	I, III
Thysanura	2-4	9, 10	21	I, III	3	II
1 <i>Japygidae</i> probably <i>Heterojapyx</i> sp						
Orthoptera	2-3	7, 9, 10	37	I, III	6	I
1 <i>Gryllidae</i> —all eggs						
Dermoptera						
1 <i>Forficulidae</i>	2		1	II		
Rhynchota						
1 <i>Lygaeidae</i>	11		4	I, III	3	I
II. NEMATODA	2-4	6-11	53	I-III	7	III
III. OLIGOCHAETA	2		2	III		
IV. CRUSTACEA	2, 3	8-9	6	I, III	2	II
V. MYRIPODA	2		1	II		
VI. ARACHNIDA	2-5	10	14	I-III	2	I, II

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THE EFFECT OF AMMONIUM ON THE POTASSIUM CONTENT OF UNSTRIATED MUSCLE AND ITS RELATION TO THE CONTRACTION PRODUCED ON WITHDRAWAL OF CHEMICAL SUBSTANCES FROM AROUND THE MUSCLE

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AMMONIUM is known to penetrate cells and replace potassium. Ammonium has some interesting effects on unstriated muscle, it produces a contraction on withdrawal (Singh, 1939b). It was assumed that ammonium penetrated the cells, and that the contraction produced by its withdrawal was due to the presence of ammonium ions within the cells. The present experiments were performed to test the validity of this assumption, and to correlate the effect of ammonium on the responses of unstriated muscle with its effect on the potassium content of the latter.

The potassium content of the muscle was determined by the method described by Cummings (1939). The frog stomach muscle (from *Rana tigrina*) was divided into two nearly equal parts, one piece was soaked in the experimental and the other in the control solution. The values were recorded as mg/g of dry as well as wet weight of muscle.

RESULTS

The potassium content of the unstriated muscle from six frog stomachs varied from 2.25 to 3.5 mg/g wet, there being a considerable variation from animal to animal. The potassium content of 6 pieces from dog stomach varied from 2.15 to 3.12 mg/g. There is thus no significant difference between the potassium contents of unstriated muscle from dog and frog stomachs, though the ionic concentration of their media differs. This is in agreement with results of some previous workers. Thus, the potassium content of ox stomach is 3.65 mg/g and that of ox retractor penis 2.67 mg/g (Constantino, 1911), that of frog stomach muscle from 3.6 to 3.43 mg/g (Meigs and Ryan, 1912). The potassium content of the striated muscle of pig is 2.5 mg/g, that of ox 3.6 mg/g. (Katz, 1896), that of cat about 3.5 mg/g (Fenn, 1938) that of rat about 3.5 mg/g (Fenn and Cobb, 1936), that of frog

striated muscle, about 3.5 mg/g (Katz, 1896, Meigs and Ryan, 1912, Fenn and Cobb, 1936).

The dog stomach muscle is therefore relatively richer in sodium than frog muscle; the former also exhibits greater tone than the latter (Singh, 1940). This is in agreement with the view previously mentioned that the greater sodium content of unstriated muscle, compared to that of striated muscle is related to greater tone of the former (Singh, 1938a). The dog retractor penis shows greater tone than dog stomach and it is significant to note that the potassium content of ox retractor penis is less than that of ox stomach and the sodium content correspondingly greater (Constantino, 1911).

The effect of ammonium on the potassium content of the unstriated muscle of dog and frog stomachs is shown in Table I. In these experiments

TABLE I

No of experiments	Nature of Muscle	No of pairs	Nature of solution	K, mg/g wet weight	Percentage change in experimental solution
1	Dog Stomach	6	Unsoaked Saline	2.67 ± 0.04 0.41 ± 0.03	- 84
2	Do	6	Saline 0.123 M NH ₄ Cl	0.28 ± 0.02 0.27 ± 0.02	- 0.03
3	Frog Stomach	5	Unsoaked Saline	2.41 ± 0.16 1.65 ± 0.04	- 39
4	Do	8	Saline 0.03 M NH ₄ Cl	1.12 ± 0.22 0.62 ± 0.24	- 44
5	Do	7	0.03 M NH ₄ Br 0.03 M N(CH ₃) ₃ Br	0.29 ± 0.04 0.76 ± 0.03	+162
6	Do	6	0.03 M N(CH ₃) ₃ Br 0.03 M NH ₄ Cl, pH 7	1.55 ± 0.47 1.09 ± 0.38	+435
7	Do	7	0.03 M NH ₄ Cl, pH 8 K-free Saline, pH 8 K-free Saline, pH 6	0.61 ± 0.38 1.14 ± 0.02 0.39 ± 0.15	- 44

only physiological concentrations of various ions have been employed; these concentrations have been used to study their effect on the responses of unstriated muscle (Singh, 1939, 1940).

In frog muscle there is a significant difference between the potassium contents of muscle soaked in normal and ammonium salines, so that ammonium enters the cells and replaces potassium. In frog muscle such a concentration of ammonium produces a withdrawal contraction, having properties of the A C contraction, which appears to be produced by ions within the muscle fibres (Singh, 1938b, 1939b).

In dog stomach withdrawal of ammonium produces no such contraction and the remarkable fact was observed, that there was no significant

difference between the potassium content of dog muscles soaked in normal and ammonium salines; besides the concentration of ammonium employed to soak the dog muscle was 0.123 M NH_4Cl , and for frog muscle it was only 0.03 NH_4Cl . Withdrawal of the latter, concentration of ammonium produces no response in dog muscle.

More ammonium replaces the potassium in alkaline than in acid solutions; this is comparable to the effect of pH on the potassium content of frog gastrocnemius (Fenn and Cobb, 1935). The loss of potassium from the muscle in a potassium-free solution is greater in acid than in alkaline solutions (Table I) as is found with the frog gastrocnemius. The ammonium withdrawal contraction is greater in acid than in alkaline solutions, suggesting that the contraction is due to the outward passage of ammonium ions.

Withdrawal of tetra-ammonium salts $\text{N}(\text{CH}_3)_4\text{Br}$, $\text{N}(\text{C}_2\text{H}_5)_4\text{Br}$ produces no contraction, which has the properties of the A.C. contraction, the permeability of the muscle to these salts is also less than that to ammonium (*cf* Ing and Wright, 1931). Withdrawal of these salts, however, appears to produce a tonic contraction, as the relaxation of the muscle on withdrawal of the tetramethyl ammonium bromide is very slow and is practically devoid of intermittent contractions.

Though no actual contraction has been observed on withdrawal of these tetra-ammonium salts, this slow relaxation is in reality a tonic contraction, as suggested by the following observations (1) The contraction produced on addition of tetramethyl ammonium bromide is intermittent so that the contraction on withdrawal of the salt is not merely due to its diminishing concentration when normal saline is added, resulting in a diminution of the stimulus (2) Tone is antagonistic to spontaneous contraction. Withdrawal of these salts immediately suppresses the spontaneous contractions in frog stomach, guinea-pig uterus, subsequently the spontaneous contractions are enhanced before return to normal. Withdrawal of thiocyanate which may result in a tonic contraction, also suppresses the spontaneous contractions and the response to A.C. in frog muscle, these latter contractions having similar properties and being antagonistic to tonic contractions (*cf* effect of withdrawal of caffeine on the response to A.C. in *Mytilus* muscle Singh, 1938b) (3) The stimulating power of these salts varies in the order $\text{N}(\text{CH}_3)_4\text{Br} < \text{N}(\text{C}_2\text{H}_5)_4\text{Br} < \text{NH}_4\text{Br}$, the rate of relaxation of the contractions produced by these substances also varies in the same order. It has been shown previously that contractions produced by withdrawal of thiocyanate, nitrate, ammonium in dog muscle are tonic and their magnitude varies similarly as the initial contractions produced by these substances.

That such a slow relaxation on withdrawal of a substance is a tonic contraction is shown by the following experiments. Withdrawal of bromide does not produce a tonic contraction, withdrawal of nitrate, thiocyanate and ammonium produces such a contraction, the potency of these substances being in the order $\text{NO}_3 < \text{SCN} < \text{NH}_4$. The rate of relaxation of the contractions produced by these substances also varies in the same order, further these substances may either produce a contraction on withdrawal or may render the relaxation slow, and it is possible to pass from a stage of slow relaxation to that of contraction on withdrawal. The slow relaxation on withdrawal of thiocyanate is thus a less powerful contraction than that produced on withdrawal of ammonium. The above phenomena are very interesting, as the cessation of electrical stimulation may also either result in slow relaxation or in tonic contraction, the slow relaxation being an intermediate stage between rapid relaxation and a tonic contraction (Singh 1938c, 1940).

Slow relaxation of a tonic contraction is also due to persistence of the contraction though the stimulating substance is withdrawn, the tonic contraction probably results in increased viscosity, as the rate change of length is diminished, and this increase in viscosity persists. The excitability of the muscle during such a contraction is greatly diminished, adrenaline antagonises such a state of muscle. In *Mytilus* muscle barium chloride produces such a contraction, all substances that produce tonic contraction, produce such a state of the muscle to some degree so that the contraction produced on withdrawal of the thiocyanate or ammonium is not very marked, if the muscle already enters into a tonic contraction, resulting in diminution of excitability. Such a tonic contraction may be prevented by previous treatment with adrenaline. Tonic contraction produced by A C in *Mytilus* muscle has similar properties (Singh, 1940).

Thus both with electrical and chemical stimulation, slow relaxation of a contraction is due (1) either to after-stimulation or (2) to structural changes, probably attended with increase in viscosity.

Increase in osmotic pressure of the medium increases the concentration of potassium within the fibres. Thus the concentration of potassium in 4 frog muscles immersed for 18h in saline of the same osmotic pressure as that of 0.120 M NaCl was $1.01 \pm$ mg/g, and in 4 muscles immersed in saline, the osmotic pressure of which was that of 0.154 M NaCl., the concentration of potassium was $1.36 \pm$ mg/g. The ammonium withdrawal contraction is augmented if the osmotic pressure is increased to 1.3-1.4 times normal; this is in agreement with the view that the ammonium withdrawal contraction is due to ions within the cells, as increase in osmotic pressure would increase the concentration of ammonium within the muscle fibres.

With frog muscle, if the concentration of ammonium in the saline is increased beyond 0.03 M NH_4Cl then the ammonium withdrawal contraction is depressed. With dog muscle, which is acclimatised to a higher concentration of sodium chloride, the ammonium withdrawal contraction can be produced if all the sodium is replaced with ammonium, the optimum concentration being 0.123 M NH_4Cl . This shows that the diminution of the withdrawal contraction in frog muscle with high concentrations of ammonium is due to a secondary depressant effect of the latter (Singh, 1939 *b*)

DISCUSSION

Facts which suggest that the contraction of frog muscle on withdrawal of ammonium is due to the outward passage of ammonium ions are (1) soaking of frog muscle in ammonium-rich saline results in a greater loss of potassium than soaking in normal saline (2) With dog muscle, which shows no contraction on withdrawal of ammonium having the properties of the A C contraction, there is no significant difference between the action of ammonium-rich and normal salines (3) There is greater loss of potassium from the muscle in a potassium-free saline at pH 6 than at pH 8, but in the presence of ammonium, there is greater loss at pH 8 than at pH 7. The ammonium withdrawal contraction is greater in acid solutions than in alkaline solutions, suggesting that it is due to the outward passage of the ions (4) There is greater loss of potassium in ammonium-rich saline, than in saline containing tetra-ammonium salts

It has been mentioned previously (Singh, 1939 *b*), that one kind of adaptation is associated with penetration of the ions into the cells; this agrees with the present analysis, as adaptation to ammonium is more rapid than to the tetra-ammonium salts

SUMMARY

(1) In frog stomach muscle, ammonium enters the cells and replaces potassium; this is associated with the ammonium withdrawal contraction having the properties of the A C contraction

(2) In dog muscle there is no significant difference between the potassium contents of muscles soaked in normal saline and in ammonium-rich saline respectively, with this is probably associated the absence in dog muscle of the ammonium withdrawal contraction having the properties of the A.C contraction

(3) Ammonium causes greater replacement of potassium than the tetra-ammonium salts, with this is probably associated the fact that the latter do not produce a contraction on withdrawal, similar to that of ammonium in frog

muscle Withdrawal of the latter salt produces a tonic contraction similar to that produced by withdrawal of ammonium in dog muscle

(4) There is greater loss of potassium produced by ammonium in alkaline than in acid solutions; but there is greater loss of potassium in a potassium-free saline in acid solutions As the ammonium withdrawl contraction in frog muscle is more marked in acid than in alkaline solutions, it suggests that the contraction is due to the outward passage of the ammonium ion.

(5) Slow relaxation of a contraction produced by a chemical substance may be due to tonic contraction on withdrawal of the substance or to persistence of the previous contraction, probably due to increase in viscosity of the muscle

We wish to thank the late Professor A J Clark, F R S, for suggesting these analyses and Lt -Col S. S Sokhey, I M S, Director, Haffkine Institute, for providing the necessary facilities

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ON SOME INDIAN EARTHWORMS

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INTRODUCTION

LITTLE has been known hitherto of the oligochaete fauna of the United Provinces and of Central India. Arrangements for a study of the earthworms of a portion of this area centering around Allahabad were very kindly made by I. D. Caleb of this College shortly after the author's arrival from Burma. Included in subsequent pages are some of the more important taxonomic results of this survey.

Twelve new species are described, of which two belong to as many new genera. Four of these species, and the new genera, have been found only in the Gangetic Valley which appears to be more interesting zoogeographically than has been thought. In the family Ocnerodrilidae two species of *Thatonia*, a genus hitherto known only from Burma, have been found in Central India, and a species of *Malabaria*, apparently characteristic of a northern portion of the Deccan, has been collected at several localities from Manikpur Junction south to Jubbulpore. *Malabaria* has been unknown hitherto north of Malabar, except for one record at Bangalore. One species is known from Burma and China. The new species of *Ocnerodrilus* appears to be restricted mainly to the Gangetic Valley, just reaching into Central India at Manikpur Junction. On the negative side may be mentioned the fact that *Gordiodrilus*, species of which are known from Burma (2), Bangalore (1) and Travancore (1), has not been found.

The Megascolecid *Ramiella cultrifera*, hitherto known only from Burma, and Christmas Island near Java, seems to be common in both the Gangetic Valley and Central India (south to Jubbulpore). Some part of Central India or some neighbouring area, presumably is to be regarded as the original home of the species from whence it may have been carried to the east, perhaps by man.

At least as important as the erection of new species, is the solution of problems arising from the fragmentary state of our knowledge of older species. The collections involved have provided information of value in answering, or partly answering, some of these questions with regard to several older species, including some of doubtful status which were erected on juvenile, a clitellate or unique types.

Most earthworm species are known from a very few specimens, often only from a unique type or from a very short series secured at the type locality. Hence another important aspect of a survey such as the present one is the possibility of securing longer series of some at least of the forms. Study of the long series may provide information as to intra-specific variation which may be of interest and importance in several ways. First of all the study

of even a short series should obviate the attribution of taxonomic value to trivial or inaccurate characterizations, as for instance in the supposed spear-head shape of penistetal tip in *L. pusillus*. Further, such study may permit recognition as such of occasional chance variations, mutations, or abnormalities of various types. In the megadriliid section of the Oligochaeta numerous species have been erected on types that are now suspected or known to be abnormal. The status of a large majority of these species is still uncertain. Indeed there is some question as to how normal the holotype of a new species of *Lennogaster* may be but attempts to secure further material were futile.

The longer series may provide information sufficient to warrant or necessitate a revaluation of certain taxonomic criteria. In this connection the variation which has been found in number and segmental location of the gizzards in two species of the genus *Drawida* must be considered in connection with Stephenson's use of gizzards in specific diagnoses and key (1923).

Further, the longer series may permit the recognition of more or less standard variations and thus obviate the erection of unnecessary species. A good illustration of this is provided by the variant forms of the new species of *Malabaria*. Had these variants been found under certain less fortunate conditions they might well have been considered to be distinct species.

Finally the study of longer series may eventually contribute to a better understanding of the problems involved in earthworm evolution. Much has been written on this subject and of that possibly much may be beside the point, because of the very limited nature of the material that has been available. In the more recent taxonomy the emphasis has increasingly been on geographical variation which must be determined from the study of long series from different localities. Geographical variation has now been found in most groups of animals. In earthworms however such variation has been recorded only by Pickford for certain South African forms. Considerable evidence apparently indicating geographical variation in several Moniligastrid and Megascolecid species of Burma had been accumulated for several years but had not yet been analyzed at the time of the Japanese invasion when much at least of the material was lost. Nothing is certainly known with regard to geographical variation of Indian species.

The author's thanks are extended to Mr I. D. Caleb for making this work possible, and to Messrs S. N. Prashad and V. R. Jha for the collection of specimens.

SYSTEMATICS

Family Moniligastridae

Genus *Drawida* Michaelsen 1900*Drawida calebi* sp. nov**Material examined.—**

Jubbulpore, Central Provinces, October, 8 juvenile 63 acitellate and 16 citellate specimens M Matthur

Nowgong, Chhatarpur State, Bundelskand Central India, October, 6 acitellate and 6 citellate specimens S N Prashad

Satna, Rewa State Central India October 1 citellate specimen E C Pahari

Manikpur Junction, Banda district, United Provinces, September 7 juvenile, 18 acitellate and 30 citellate specimens M Matthur

Tanda Falls, Mirzapur district, U.P. October, 3 juvenile, 7 acitellate and 47 citellate specimens E C Pahari

External characteristics—Length 35–50 mm Diameter 3–4 mm Unpigmented Prostomium prolobous Setæ, ab ca = cd, aa < bc, dd ca $\frac{1}{2}$ c Nephropores on or very close to d lines, near anterior margins of segments, from iii posteriorly, functional on x, occasionally one of the pores of xi lacking or unrecognizable as a patent aperture Clitellum, light orange to pinkish, annular, on x-xiii

Spermathecal pores fairly large, transversely placed slits on 7,8 just median to c lines Male pores fairly large transversely placed slits on 10/11 at or near mid bc

— Genital markings small, nearly circular greyish translucent spots, which may be slightly concave clearly marked off from a narrow rim which may be slightly tumescent but usually is not clearly demarcated peripherally On 20 specimens selected at random from the Tanda Falls series the markings are located as follows, median (unpaired), presetal on vii (0), viii (16), ix (19), x (18), xi (19), xii (1), xiii (0), postsetal on vii (2), viii (10), ix (9), x (6), xi (0), xii (0), xiii (0); paired, occasionally almost in contact at mid-ventral line, postsetal on vii (4), viii (3), ix (1), x (2), xi (1); widely paired in bc, presetal on vii (18), viii (18), ix (1), x (0), xi (1), xii (0), xiii (0) postsetal on vii (0), viii (2), ix (0), x (20), xi (0), xii (20) xiii (0) Sites characterized above by the "(0)" may very rarely have the marking on other specimens Unpaired medians usually are not exactly placed in a straight longitudinal rank Each grey spot indicates the presence of a small spheroidal to

shortly ellipsoidal translucent gland internal to the epidermis but unrecognizable internally until after removal of longitudinal musculature

Internal anatomy.—Gizzards, two (7), three (65), or four (39), are in XII-XVII as shown below. The postgizzard portion of the oesophagus is wide, sigmoid, a short portion in one or two segments at the hind end narrowed and valvular. Intestinal origin in XXIV (4), XXV (24), XXVI (30), XXVII (13). Of eight specimens with gizzards in XII-XIV or XII-XV, in which the intestinal origin was noted the origin is in XXIV (3), XXV (4), XXVI (1). No typhlosole (10). Enterosegmental organs are represented anteriorly by a longitudinal body just lateral to the dorsal trunk on each side, of almost exactly the same colour and appearance as a blood vessel, passing at either end near the septa downwards into the gut. From the ventral face of each rod four to six smaller rodlets pass downwards into the gut. Chloragogen which is present in a thick band on each side of the dorsal vessel is lacking on the enterosegmental organs.

Location of gizzards

Segments	Number of specimens from		
	Tanda Falls	Jubbulpore	Nowgong
XII-XIV	1		
XII-XV	10	3	
XIII-XIV		2	
XIII-XV	23	9	
XIII-XVI	12	8	2
XIV-XV		1	1
XIV-XVI	4	21	4
XIV-XVII		5	
XV-XVI		1	3
XV-XVII			1

In XII-XV, Satna specimen

The last pair of hearts is in IX (113). Commissures from the extra-oesophageals are on the posterior faces of 9/10 and 8/9 but in the latter case only ventrally, the upper portions within the septum and unrecognizable without dissection. Extra-oesophageals are lateral to the hearts.

Testis sacs are about equally in IX and X. The vas deferens is rather short, in a vertical column of more or less closely crowded short loops on the anterior face of 9/10, ventral portions of the hearts of IX within the columns, in X nearly straight or in several short loose loops, passing up onto the prostate without penetrating into the parietes and disappearing on the anteromesial aspect before reaching the apex. Prostates are not quite spheroidal, usually inclined slightly anteromesially, sessile on the parietes. There is no

cœlomic glandular layer, the surface smooth and firm. The wall is rather thick but the major portion is of soft tissue, the capsule thin, translucent and whitish. The cavity is nearly filled by a rather conical body which has at the centre of flattened apex a small, transversely placed slit. This conical body is protrusible to the exterior as a shortly tubular penis which is ca $\frac{1}{2}$ mm long (two specimens only)

The ovarian chamber was opened in each dissected specimen, septa 10/11 and 11/12 apparently coming into contact laterally and dorsally just at or just internal to the parietes. Ovisacs may extend as far back as xx

The spermathecal ampulla is rather small, nearly spheroidal. The duct is four to five mm long, passing into the pointed apex of the atrium. The latter is smaller than the prostates, rather conical, always more or less erect in viii but bound to the posterior face of 7/8. In the anterior wall of the atrium there may be one or two glands like those associated with the genital markings.

Regeneration—A Nowgong worm has a short tail regenerate at 73/74

Abnormality—One worm has a spiral metamerism in region of ix-xi, so that the left male pore appears to be one segment behind the right

Remarks—Along the mid-dorsal line from the clitellum nearly to the hind end there is visible a dark streak or line. There is no median longitudinal muscle-band nor pigment and the dark appearance seems to be due to the presence of a definite gap in the longitudinal musculature

In juvenile specimens prostates appear to be fully developed but spermathecal atria are still confined to the parietes and ampullæ are rudimentary

So many Indian species of the genus *Drawida* are so inadequately characterized that there is little point in attempting to determine relationships of the form described above. Absence of a cœlomic glandular layer on the prostates at present appears to indicate relationships with the *grandis* group of species from which the Deccan form is distinguished by the numerous genital markings and the well developed spermathecal atria

Diagnosis—Male pores transversely placed slits on 10/11 at mid bc. Spermathecal pores on 7/8, just median to c lines. Genital markings small, unpaired and median or closely paired in aa, and widely paired in bc, presetal and postsetal on vii-xiii. Nephropores on or close to d lines throughout Length 35–50 mm Diameter 3–4 mm

Gizzards, 2–4, in xii-xvi; intestinal origin in xxvi (\pm 1). Vas deferens rather short, in a small column of loops in ix, nearly straight in x, passing into the anteromedian aspect of the prostate directly. Prostates with no cœlomic

glandular layer, nearly spheroidal, sessile on the parietes, with an internal ventral portion protrusible as a shortly tubular penis Spermathecal atrium conical in VIII, smaller than prostate Genital marking glands spheroidal to shortly ellipsoidal, between epidermis and longitudinal muscle

Drawida willsi Michaelsen 1907

Material Examined —

Jubbulpore, Central Provinces, October, 450 specimens M Matthur

Satna, Rewa, Central India, October, 217 juvenile, 3 presexual and 5 postsexual achitellate specimens E C Pahari

Manikpur Junction, Banda district, United Provinces, September, 604 juvenile and achitellate 17 chitellate specimens, November, 411 specimens M Matthur

External characteristics — Pigmentation blue, post-chitellar portions often with a red to purple appearance Nephropores, whenever recognizable, are in or very close to *cd*, certainly so on VII-XVII, pores on X functional

Male pores and apertures of genital marking glands minute, on or very close to *b* lines, on or in line with 10/11 and 9/10 Male porophores circular to transversely elliptical, slightly raised, sharply demarcated, extending equally on to X and XI, a small central portion (containing the male pore) slightly depressed or protuberant in a rather conical fashion and with male pore on vential end Genital markings which always appear later than the male porophores are smaller than the porophores, extending equally on to IX and X, nearly circular, merely areas of epidermal whitening or raised and disc-like but never with a central protuberance

Internal anatomy — Gizzards, 2 (29), 3 (66), or 4 (5), in XII-XVI as follows XII-XIII (1), XII-XIV (25), XIII-XIV (24), XIII-XV (36), XIII-XVI (5), XIV-XV (4), XIV-XVI (5) Intestinal origin in XXI (25), XXII (36), XXIII (15) No typhlosole Last hearts in IX (100) Extra-oesophageals lateral to the hearts

Vas deferens rather thick relative to size of worm, rather short, in several loops on the anterior face of 9/10 around the hearts, several further loops on the posterior face of 9/10, passing directly onto the prostate without first penetrating the parietes and upwards nearly to the vental end Prostates erect, capsule digitiform, perhaps slightly widened ventally Genital marking glands present in 92 specimens (lacking on one side, 8), in 91 worms are smaller than the prostates, very rarely only slightly so, occasionally only slightly protuberant into coelomic cavity, in one worm as large as the prostates The capsule is digitiform, always shorter and slenderer than that of the prostate,

the equivalence in size of glands in the exceptional specimen due to hypertrophy of the coelomic glandular layer

Spermathecal atria, except as noted below, always in vii, digitiform, as long as, to slightly longer than the prostates, erect, an ental portion occasionally slightly curved, wall thick. The spermathecal duct passes into the posterior face of the atrium close to the parietes. Ovarian chambers were invariably opened in course of dissection, in spite of considerable care.

Regeneration—Of the November Manikpur specimens 58 have tail regenerates of which the following levels were determined 36/37, 50/51, 64/65, 71/72, 73, 74, 80/81, 83/84, 87/88, 89/90, 92/93, 104/105, 112/113, 115/116 (2), 133/134, 144/145, 155/156. Regenerates are unpigmented.

Abnormality—The left spermathecal atrium of one worm is considerably thickened entally, the dorsal end deeply grooved to mark off right and left rounded lobes. One atrium of another worm is bifid entally, with one lobe protuberant into viii.

A Manikpur specimen had lost its head at 6,7 Segment vii, setæ retained, had been modelled into a dome-shape, with a small, slightly brownish, imperforate scar at the centre.

Remarks—The account above is based on the Satna specimens. Of some forty odd clitellate specimens from Manikpur, seven have no genital markings and glands, six have a marking on the right side only, four have the left marking only.

Family Ocnerodrilidae

The collections with which this article is concerned provide really long series of two species. Similar numbers have been unavailable for study hitherto in any species of this family. In one species no variation of especial significance has been detected. In the other species there is considerable variation in external characteristics, so much, in fact, that if the different variants had been secured in certain less favourable conditions they might well have been referred to three or four different species. In a preliminary identification from external characteristics one variant form would almost certainly have been considered to belong to a totally different genus. Practically all stages intermediate between the variant forms and the normal form, have however been available.

The internal anatomy of well over a hundred specimens of this variable species has been investigated, by dissection, a study which would have been prohibited by the cost and time required if made by the usual method of microtome sections. Results obtained provide some confirmation for a

suspicion, already expressed, that some at least of the confusion still prevailing in Ocnerodrilid systematics is due, in part at least, to the attempt to determine internal characteristics of taxonomic importance from more or less refractory material by the technique of the microtome. While it may at first appear to be difficult to dissect worms so small as most Ocnerodrilids are, with practice, good instruments, and under the binocular, dissection becomes easy and rapid (more easy in fact than in some larger forms that might be mentioned) of specimens down to one mm. in diameter. Dissection of individuals less than one mm. thick, down to little more than 0.5 mm., is also possible though of course more difficult.

Genus *Thatonia* Gates, 1942

***Thatonia exilis* sp. nov**

Material examined —

Nowgong, Chhattarpur State, Bundelkhand, Central India, "Black cotton soil, mixed with decaying leaves", October, 7 juvenile specimens
S N Prashad

Manikpur Junction, Banda District, United Provinces, November, 4 juvenile specimens M. Matthur

External characteristics — Length to 50 mm. Diameter 0.5–0.75 mm. Spermathecal pores are on *b* lines on 7/8, at or slightly lateral to mid *bc* on 8/9 (11). Male pores appear to be immediately in front of *b* setæ of xviii. Just median to the *a* line on each side and between the setal arcs of xvii and xix there is a nearly straight line of greyish translucence in the epidermis but no definite groove.

Remarks. — All ventral setæ of xvii–xix are present and clearly visible externally. Very slight tumescences are recognizable with some difficulty on the setal arcs of xvii and xix just lateral to the *b* lines, presumably anlage of prostatic porophores. Prostates of xvii and xix are well developed as are the spermathecae. Genital markings are quite unrecognizable as also prostate-like glands in xx or posteriorly. Spermathecal pores are not certainly recognizable externally, the locations confirmed by dissecting spermathecal ducts out of the parietes.

T. exilis is distinguished from other species of the genus by location of the posterior spermathecal pores at or lateral to mid *bc*. An unnamed species of *Malabaria* from Bangalore is similarly distinguished from other species of its genus.

Thatonia parva sp. nov*Material examined* —

Satna, Rewa State, Central India, October, 1 clitellate specimen E C Pahari

Nowgong, Chhattarpur State, Bundelskand, Central India, "Black cotton soil, mixed with decaying leaves", October, 1 juvenile specimen S N Prashad

Daraganj, Allahabad, United Provinces, September 1 achtellate specimen M Matthur

External characteristics — Length 24 mm Diameter 1½ mm Prostomium epilobous, tongue open Ventral setæ of iii-xii appear to be slightly larger than posteriorly but apparently are not especially enlarged on viii-ix Spermathecal pores on or immediately lateral to *b* lines on both 7/8 and 8/9

The genital shield is transversely placed extending well into *bc* raised, apparently an area of marked epidermal thickening Seminal grooves are rather wide, shaped like parentheses, the concave sides laterally, ends deepened along setal arcs of xvii and xix in *ab* Ventral setæ of xvii-xx lacking, male and prostatic pores unrecognizable

On xx there is a transversely placed, smooth-surfaced area of less marked epidermal thickening, not clearly demarcated from the male field at site of 19/20 On this area and in *ab*, on each side, there is a transversely placed narrowly elliptical depression, in which a pore is not certainly recognizable

Internal anatomy — The gut is widened and moniliform in viii-ix, the wall thickened but not especially so ventrally, and of a uniform dark red colour In x the wall is less thickened and discrete, vertical, red striations are recognizable There is no median groove on the floor of the gut in these segments

The vasa deferentia pass into the parietes in line with the prostatic ducts but quite definitely nearer to the anterior ducts, approximately over site of 17/18 Prostates, including ducts, are about ten mm long, the ducts slender and long enough to reach backwards through four segments Genital marking glands are of the same size and appearance as the prostates

The spermathecal duct is circular in cross-section, thick-walled, reaching only shortly into the coelomic cavity, with narrow lumen (filled with iridescent material presumably spermatozoa). The ampulla is thin-walled, transparent, collapsed except entally where it is distended by a small mass of white material, much longer than the duct, elongately saccular, but even at the ental end not much wider than the duct.

Remarks—The type appears to be fully sexual, with spermatozoal iridescence in the spermathecal duct and a brilliant iridescence on the male funnels.

Nowgong and Allahabad specimens at present appear to be referable to *parva* by location of the spermathecal pores in spite of absence of genital markings of *xx* and their glands. The juvenile specimen has no male field or seminal grooves though prostates and spermathecae are well developed. The Allahabad worm likewise has no male field or seminal grooves and ventral setae are present on xvii-xx. Nevertheless male funnels are characterized by a brilliant spermatozoal iridescence. At 53/54 there is a tail regenerate 1½ mm long, of 11 setigerous segments and a large growth zone. The first three or four segments may possibly be a head regenerate.

Except as has been noted above the anatomy is as in *T. gracilis* Gates 1942 from which the present species is distinguished as follows by the shape of the male field, by the crescent or parenthesis shape of the seminal grooves, absence of marked enlargement of ventral setae of viii-ix, presence of a genital marking on *xx* associated with prostate-like glands, and possibly also by a more anterior location of the male pores.

By the presence of a pair of prostate-like, genital marking glands in *xx*, this species parallels *paludicola* Stephenson 1924 in the genus *Malabaria*. In the genus *Eukeria*, *peguana* Gates 1942 has a similar pair of genital marking glands but in *xxi*.

Genus *Malabaria* Stephenson, 1924

Malabaria sulcata sp. nov.

Material examined.—

Manikpur Junction, Banda District, United Provinces, November, 75
juvenile 713 achtellate and clitellate specimens M Matthur

Jubbulpore, Central Provinces October 4 achtellate and 1 clitellate
specimens M Matthur

Satna, Rewa State Central India, October, 1 achtellate specimen E C
Pahari

Nowgong, Chhatarpur State, Bundelkhand, Central India, "Black cotton
soil mixed with decaying leaves", October 1 achtellate specimen
S N Prashad

External characteristics—Length 27-55 mm Diameter 0.6-1.5 mm
Number of segments 97-111 Unpigmented Prostomium epilobous,
tongue open posteriorly Setae begin on ii, paired, a and b of xvii lacking.
Clitellum saddle-shaped, extending from 12/13, on xiii, or 13/14 to 20/21.

onto xxi, or to 21/22, reaching ventrally to *b* lines, recognizable externally only by a faint yellowish appearance and indistinctness or absence of intersegmental furrows. On xix-xxi the faint yellowish colouration may characterize all of the segment but the ventral epidermis does not appear to be thickened.

Spermathecal and female pores are on the *b* lines

Male porophores are sharply demarcated, raised, flat surfaced discs of shortly elliptical outline, areas of considerable epidermal thickening, reaching to or nearly to 16/17 and 17/18, laterally to mid *bc*, or slightly further, separated from each other mesially by a small space of about the same width as *ab*, slightly diagonal, convergent posteriorly. On each porophore there is a fairly deep seminal groove, also slightly diagonal, the anterior end lateral to the *b* line, the posterior end about at the *a* line, with prostatic and male pores in the deepened anterior and posterior ends of the groove.

Internal anatomy—Septa 5/6-9 10 are strengthened. The ventral surface of the gut in ix and x may have a perfectly smooth, regularly convex surface, in which case the lumen may be horizontally slit-like in cross-section midsegmentally due to the presence of a conspicuously raised plateau on the floor of the gut. Or there may be midsegmentally in each of ix and x, on the ventral face of the gut a rather conspicuously raised protuberance that looks like an unpaired pouch. In other specimens no specially marked off protuberance is recognizable, either externally on the ventral face of the gut or internally on the floor, though the ventral wall is of course thickened. In any case there are recognizable, even in dissections, two distinct cavities, each widened ventrally, in the thickened floor of the gut, in each of segments ix and x.

Metandric, seminal vesicles usually reaching into xiii, occasionally into xiv or xv. Prostates are long and may extend back into 1. Prostatic ducts pass into the parietes at *b* lines about midsegmentally. Conspicuously protuberant into the coelomic cavity of xviii on each side is a pyriform body which narrows ectally to a delicate stalk that passes through 17/18 close to the parietes and then into the body wall at the *a* line. The wall is thick, the lumen slit-like in cross-section. The male deferent duct which is at first lateral to the prostatic duct turns mesially in a posterior portion of xvii and apparently unites with the slender stalk of the pyriform body just at or within the parietes.

Spermathecae are elongate, coiled in ix, or ix-x, or extending posteriorly, occasionally into region of xviii-xxiv. A short ectal portion, about as long as the interval *bc*, is smooth, opaque, circular in cross-section, with thick

wall and narrow lumen. The rest of the spermatheca has a thin, almost transparent wall and may perhaps be called irregularly tubular but is collapsed, irregularly folded and constricted. Occasionally a short, ovoidal to ellipsoidal, terminal portion is definitely constricted off, though scarcely widened, and looks somewhat like an ampulla.

Regeneration—Short tail regenerates have been noted at the following levels 27/28, 33/34, 35/36, 38/39, 41/42, 43/44, 46/47, 47/48, 48/49, 49/50, 52/53, 53/54, 54/55, 72/73, 94/95

Variation—One worm only has more than two male porophores,—in addition to the usual porophores on xvii, an extra but normal porophore each on xv and xvi, on the right side. Associated with each porophore is a prostate but on the right side only the porophore of xvi is associated with a pyriform body.

Four worms have one of the two male porophores on xvi rather than xvii, on the left side (2 specimens), on the right side (2).

Of the remaining specimens 111 have male porophores that diverge from the norm described above. Variant porophores are of three types (1) Porophore transversely placed, rather than diagonally, confined to xviii, seminal groove crescentic, transversely placed, concave posteriorly or V-shaped, the apex anteriorly (2) Porophore diagonally placed, but with anterior end nearer the midventral line, the posterior end in *b*, at, just in front of, or just behind the setal arc of xviii, seminal groove more or less definitely question-mark shaped, with bottom end of mark posteriorly and laterally. Ventral setæ of xviii are usually present but one or both may occasionally be absent, on one side or the other, never on both sides (3) Porophore longitudinally placed, dumb-bell-shaped, seminal groove about on the *b* line, the major portion straight, a short anterior portion always bent mesially. A very short posterior portion may occasionally be bent laterally at a sharp angle. This type of porophore usually reaches from setal arc of xvii nearly to setal arc of xix, with ventral setæ of xvii-xviii lacking, but may reach only onto xviii, with *a* and *b* of xviii present or lacking according as to whether the porophore ends just in front of setal arc or on setal arc. On one worm the right porophore reaches nearly to 19/20 while the left nearly reaches the setal arc of xx, ventral setæ of xix also lacking. The frequency of the different variants is indicated in the tables below.

Each worm with variant porophores has been dissected. One specimen with the left porophore transverse and the other diagonal, lacks a pyriform gland on the left side. Otherwise the relationships of prostate glands,

Both porophores the same

Characterization	Number of specimens
Normal	
Transversely placed	602
Seminal grooves crescentic 7	10
Seminal grooves V-shaped 2	
Left groove crescentic, right V-shaped, 1	
Diagonally placed	6
Longitudinally placed	12
Total	630

Both porophores not the same

Characterization	Number of specimens
One porophore normal	
Variant porophore transversely placed, 3 on left side (2), right side (1)	61
Variant porophore diagonally placed, 26 on left side (16), right side (10)	
Variant porophore longitudinally placed, 32 nearly to level of setal arc of xviii (a and b of xviii +) right side (1) left side (2), to setal arc of xviii (a and b of xviii 0) left side (4) right side (2), nearly to setal arc of xix (a and b + on xix but 0 on xviii) left side (13), right side (5), to setal arc of xix (a and b of xix 0), left side (2) right side (2), nearly to 19/20 (a and b of xvii-xix 0) right side (1)	
Neither porophore normal	
One transversely placed, the other diagonally placed	5
Left transverse right diagonal, 4	
Left diagonal, right transverse 1	
One transversely placed, the other longitudinally placed	3
Left transverse right longitudinal, 1	
Left longitudinal, right transverse, 2	
One diagonally placed, the other longitudinally placed	13
Left diagonal, right longitudinal, 5	
Right diagonal, left longitudinal 8	
Total	82

male deferent ducts and pyriform glands to xvii are always normal, as in twenty specimens with normal porophores

One variant specimen remains to be considered. On this worm the right porophore is longitudinally placed, extending from 16/17 well onto xviii, the seminal groove straight, with no bends or hooks anteriorly or posteriorly. The right porophore is diagonal, reaching well onto xviii. Male terminalia are normal on the right side. On the left side the vas deferens and pyriform body are normal but there is no prostate anteriorly in xvii. Instead two prostates, closely interwound, are present behind the pyriform body.

Ectally the prostates separate, one passing into the parietes immediately behind the pyriform body, the other passing into the parietes somewhat in front of 18/19

Remarks—No spermatozoal iridescence was noted on male funnels or in spermathecae, even of clitellate specimens

Except as noted above, and for the absence of ovaries in XII, of testes and male funnels in IX and X of clear gland genital markings, the anatomy is like that of *M. lewis* (for which *vide* Gates, 1942, p. 92)

M. sulcata is distinguished from all other species of the genus and, in particular, from the only other metandric species *M. oryzivorus* (Stephenson), 1924, by the sulcate male porophores and the pyriform bodies that open, along with the male deferent ducts, into the seminal grooves

Diagnosis—Male porophores diagonally placed convergent posteriorly, bearing seminal grooves containing prostatic pores anterolaterally and male pores (conjoined apertures of deferent ducts and pyriform bodies) postero-medially. Spermathecal pores on or close to $\frac{1}{2}$ lines. Length to 55 mm. Diameter to 1½ mm.

Metandric, seminal vesicles in XII. A pyriform body, protuberant into coelomic cavity of XVIII, associated with each male deferent duct. Spermathecae elongate

Malabaria sp.

Material examined—

Daraganj, Allahabad, United Provinces, September, 9 juvenile and 12 ac clitellate specimens December, 3 ac clitellate specimens M Matthur

Remarks—Length 40–60 mm. Diameter to 1 mm. Holandric, with seminal vesicles in XI and XII

None of the worms are sexual and all are abnormal, athecal and aprostatic or if prosthetic with only one gland present. Prostates, when present, are well developed, usually long enough to reach into XXX, in one specimen 13 mm long

Although careful search has been made every month for a period of over a year in various Daraganj localities similar to that where the specimens were obtained, no further worms have been secured

These notes are included to emphasize some of the difficulties involved in work on the Oenoperdrilids and to indicate the northernmost occurrence of the genus *Malabaria* in India

Genus *Ocnerodrilus* Eisen 1878Subgenus *Ocnerodrilus* Eisen*Ocnerodrilus tenellulus* sp. nov.

1942 *Ocnerodrilus occidentalis* (part) Gates *Bull. Mus. Comp. Zool. Harvard*, LXXXIX p. 99 (June specimen from Rangoon only)

Material examined —

Daraeanj Allahabad United Provinces, "drains", June, 3 achtellate and 38 clitellate specimens; July, 1 juvenile, 3 achtellate and 302 clitellate specimens; August, 10 juvenile and 1 clitellate specimens; September 47 achtellate and 247 clitellate specimens; October 55 clitellate specimens; November, 22 clitellate specimens; December, 125 achtellate and 24 clitellate specimens; January, 2 clitellate specimens; February 9 clitellate specimens; March, 46 clitellate specimens; April, 2 achtellate and 91 clitellate specimens.

Muthiganj, Allahabad, "Earth around roots of potted plants", January 2 clitellate specimens; "vegetable debris at bottom of small, concrete, water tank", July, 4 achtellate and 4 clitellate specimens.

Allahabad, "Banks of McPherson Lake", September, 2 achtellate and 39 clitellate specimens; "Municipal dump, 1 cedar Street", September, 39 achtellate and 113 clitellate specimens.

Naini, Allahabad district, August, 3 clitellate specimens.

Manikpur Junction, Banda District, U.P., September, 2 achtellate and 1 clitellate specimens; M. Matthur

Madhosingh Junction, Benares State, U.P., September 1 achtellate and 1 clitellate specimens; M. Matthur

Partabgarh Partabgarh district, U.P. September, 101 clitellate specimens; M. Matthur

Fatehpur Fatehpur district, U.P., September, 1 achtellate and 11 clitellate specimens; M. Matthur

External characteristics — Length 12–20 mm (Greatest length when fully extended 40 mm.) Diameter to 1 mm. Unpigmented, body wall (except for clitellum) of living specimens transparent. Postostomium slightly epilobous, tongue open. Setae paired, *a* and *b* of xvii always present. Clitellum, gold, orange or reddish in living specimens, light yellowish preserved, annular, extending from a posterior portion of xiii or 13/14 to 19/20, the presetal or postsetal furrow on xx, intersegmental furrows lacking, setae present.

Female pores transversely placed slits on or just lateral to *b* lines. Male pores about on setal arc, slightly lateral to *b*. There are no porophores and pores usually are not definitely recognizable though positions are indicated by very small spots of marked white opacity which may be very slightly protuberant in a rather conical fashion.

Internal anatomy—Prostates are short, transversely placed, usually long enough to reach only into *cd*, occasionally long enough to reach to the mid-dorsal line.

Abnormality—Left prostate, about twice the usual size, opens to exterior on 15/16 about on the *b* line (1).

Remarks—Juvenile specimens 5-7 mm long and *ca* 0.5 mm thick, apparently have fully grown prostates.

Except as noted above the anatomy is as in *O. occidentalis* (*vide* Gates, 1942, p. 99).

All forms referable to the subgenus *Ocnerodrillus* have hitherto been included in one species. A single specimen from Rangoon, very similar (if not identical) to those described above was originally suspected (Gates, 1942, p. 100) of being "only a slightly abnormal specimen of *occidentalis*" the abnormal condition possibly due to inhibition of development of male porophores and of full development of prostates, associated with a failure to shed the ventral setæ of xvii. In view of the total lack of variation with respect to the characteristics concerned, in a large number of specimens, and throughout the whole year, it now seems preferable to regard the variant form as a distinct species.

Diagnosis—Male pores just lateral to *b* lines, on setal arc, no porophores, ventral setæ of xvii present. Length 12-20 mm. Diameter 1 mm. Prostates short.

Distribution—Known at present only from four districts in the southern portion of the United Provinces, and also from Burma, but probably distributed as widely around the world as *O. occidentalis*.

Family Megascolecidae

Genus *Plutellus* E. Perrier, 1873

Plutellus exilis sp. nov

Material examined—

Daraganj, Allahabad, United Provinces, July, 3 acitellate specimens; September, 89 juvenile specimens, November, 47 juvenile, 29 acitellate and 7 chitellate specimens, December, 71 juvenile, 84 acitellate and

35 clitellate specimens, January, 7 ac clitellate and 37 clitellate specimens, February, 2 clitellate specimens. March, 1 clitellate specimen. April 60 juvenile or ac clitellate specimens and 1 clitellate specimen.

External characteristics — Length 60–70 mm. Diameter 1–2 mm. Unpigmented, clitellum rather greyish. Clitellate specimens are hooked or curved ventrally in clitellar region so that anterior portion of the axis is J-shaped. Prostomium epilobous, tongue open. Dorsal pores not certainly recognisable though probably present at least posterior to the clitellum. Setæ begin on ii on which all are present, closely paired, ab ca = cd, aa < hc, dd ca = $\frac{1}{2}$ C. Clitellum saddle-shaped, markedly protuberant, reaching ventrally almost to b lines or even into ab and anteroposteriorly to 13/14 or slightly onto xiii and to 18/19 or slightly onto xix, intersegmental furrows lacking.

Quadrithecal, spermathecal pores minute, on 7/8–8/9, on, just or slightly lateral to h lines. Female pores (paired) just behind site of 13/14, much nearer to a lines than to each other, on a transversely placed, presetal area of marked epidermal thickening of same appearance as clitellum with which it may be continuous laterally. Male pores, not actually seen, minute, about on b lines, in slight depressions on ventral faces of very small, rather indefinite protuberances.

The male field is usually clearly demarcated, slightly raised, shortly and thickly elliptical in outline, usually longitudinally, rarely transversely placed, reaching laterally to clitellar margin and anteroposteriorly slightly onto xvii and xix. Within the male field, in addition to the male porophores, there is always at least one genital marking. Locations (on 81 specimens) are as follows — median, presetal (7), occasionally an additional median on the setal arc, 8), on the setal arc (2) or postsetal (8), paired, presetal, in front of, anterolateral or anteromedian to the male porophores (33).

Postclitellar markings are, like those within the male field, small raised tubercles of circular outline, clearly marked off into a greyish translucent central area and an opaque, narrow rim, in ab or with centres about on the h lines, over sites of intersegmental furrows. Each pair of markings is included within a clearly demarcated, transversely placed, slightly raised area of less epidermal thickening than the tubercles. As indicated below one of a pair is often lacking in which case the field may be slightly asymmetrical. Fields bearing markings on 17/18 and 18/19 are not clearly marked off from the male field. Preclitellar markings, occasionally present, are either not included in definite fields of epidermal thickening or else the fields are less distinctly marked off. On 130 specimens the markings are located

as follows, 16/17 (1, right side), 17/18 (4), 18/19 (1, left side), 19/20 (14, left side 5, right side 2), 20/21 (76, left side 12, right side 13), 21/22 (11, left side 3, right side 1), postsetal and median on viii (1), on ix (19), presetal and paired on ix (5). In this series 31 specimens have no markings aside from those on the male field, while five have two pairs of postclitellar markings.

Internal anatomy—Septa 5/6-8/9 muscular, attached to parietes, at least ventrally, anterior to the corresponding intersegmental furrows

The portion of the oesophagus in front of 5/6 is widened, whiter and stronger than other parts of the oesophagus but obviously is not a well-developed gizzard (20). The rest of the oesophagus is slender, valvular in region of insertion of 13/14 (19), the intestinal origin in xiv just behind 13/14 (19). In one worm the origin apparently is midsegmentally in xiii, 13/14 inserted in a slight constriction. No typhlosole. On each side of the gut midsegmentally in vii-v there is an anteroposteriorly flattened gland (?) increasing in size anteriorly.

The dorsal blood vessel (single) is continued anteriorly onto the pharyngeal bulb. A supra-oesophageal is recognizable only in x-xii. Extra-oesophageals have not been found. Lateroparietal trunks, usually distended with blood, are recognizable in xxiv-xiii, in the latter segment passing off from the parietes and onto a longitudinal suboesophageal mesentery. No subneural. Hearts of x-xii are latero-oesophageal (last pair in xii, 20). Commissures of ix apparently are lateral.

Holandric, testes and male funnels apparently free, seminal vesicles acinous, in xi and xii (20). Prostates are usually confined to xvii-xviii but may extend into xx or rarely even into xxi. The duct is short, slender and passes into the parietes posteriorly in xviii. The male deferent duct passes into the anterior face of the prostatic duct just as the latter enters the parietes. The two penisetal follicles are bound together ventrally but diverge ectally, one passing into the parietes over the *a* line the other over the *b* line on the posterior face of the prostatic duct. The ental portion of the setal shaft is practically straight but the ectal fourth to third usually is strongly curved. The tip usually is bluntly rounded (worn?) but may be sharply pointed. At margins of the shaft behind the tip are several slight incisions perhaps indicating presence of triangular teeth. Length 0.35-0.45 mm., maximum diameter 5-8 μ .

The spermathecal duct is about as long as the much wider ampulla from which it is clearly marked off externally. The lumen of the duct is small but may be widened ental to the diverticular junction. The diverticulum

which is a little more than half the length of the duct may be slenderly club-shaped, with thick wall and narrow lumen in the very slightly narrowed ectal half or the ental portion may be more definitely marked off externally as a spheroidal to shortly ovoidal seminal chamber. The diverticulum passes into the duct about half-way towards the ampulla or just in the ental half, apparently on the median face.

Glandular material has not been found over sites of genital markings, either on the parietes or within the musculature.

Remarks—Most of these worms have come into the laboratory in poor condition. The few that have been complete and apparently in good condition coiled up more or less tightly when killed.

P. exilis is distinguished from all Oriental species of the genus *Plutellus*, except *sikkimensis* Michaelsen 1910, by the intestinal origin in xiv, and from *sikkimensis* by the quadrithecal battery, location of spermathecal pores on or lateral to b lines, absence of seminal vesicles in ix, etc.

Genus *Perionyx* E Perrier, 1872

Perionyx festivus sp nov

Material examined—

Jubbulpore, Central Provinces, October, 376 juvenile specimens February, 339 juvenile specimens M Matthur

External characteristics—Length to 70 mm. Diameter to 3 mm. Pigmentation red, restricted to the dorsum throughout, ventral margin of pigmentation even and sharply demarcated. Prostomium epilobous, tongue always open behind. Setal circles without definite, regular, mid-dorsal and ventral gaps. Nephropores, at least behind clitellar region, irregularly alternate between pigmented dorsum and unpigmented ventrum, dorsal pores 4–6 intersetal intervals above margin of pigmentation, neither dorsal nor ventral pores in really regular longitudinal ranks. First dorsal pore on 3/4 (82, the marking on this furrow often smaller than behind and may not always indicate a functional pore), 4/5 (118), or 5/6 (10).

Internal anatomy—Septa behind clitellar region thickened. Gizzard small but definitely muscular, in v. Intestinal origin apparently in xvii. No typhlosole. Last hearts in xiii (20). Nephridia large (behind clitellar region filling most of the coelomic spaces to or nearly to level of dorsal face of gut) and without bladders.

Regeneration—Normal tail regenerates were noted at the following levels 40/41, 43/44 (2), 48/49, 51/52, 54/55, 60/61, 64/65, 68/69, 71/72, 72/73,

73/74, 77/78, 83/84, 88/89, 101/102, 111/112, 125/26, 135/136, 155/156 One of these substrates is the smallest specimen secured and most of the substrates are from the smaller specimens Twelve posterior substrates have head regenerates at unknown anterior levels, the heads of 12 segments (1), 13 (1), 14 (2), 15 (2), 16 (1), metameric differentiation incomplete (5). Other posterior substrates have head regenerates as follows. 7 segments at 7/8, 6 segments at 9/10, 10 segments at 11/12, 15 segments at 14/15 A shorter substrate has a head regenerate of 15 segments at 15/16, and a tail regenerate at 39/40 Such double regenerates are only rarely found

Two anterior substrates have abnormal posterior regenerates, one a caudal monstrosity at 65/66, the other a bifid regenerate at 111/112, both branches normal tails

In addition many of the larger specimens, while showing no definite external evidence of regeneration, do have internal indications of regeneration of anterior portions This has made more difficult recognition of taxonomic characteristics

Shape of prostomium and location of first dorsal pore is characteristic on regenerates and enables immediate distinction of a head regenerate of this species from one of *sansibaricus*, the only other species of *Perionyx* from the type locality

Abnormality—Assuming that the two clusters of possible penial setæ (*vide under Remarks*) indicate approximate sites of the male pores, segmental location was determined on a hundred of the smaller specimens, with the following results, male pores on xvi (1), xvii (3), xviii (81), (xix) (2), xx (2), xxii (1) On the other ten specimens there are two to five pairs of male pore areas, on two or more successive segments of xvii-xxii. In *P. excavatus* which is a penisetal species, similar conditions develop in substrate segments following regeneration of a new head at or behind 15/16

Remarks—Genital apertures, even rudiments thereof, are unrecognizable, except on one worm, the largest, which has closely paired spermathecal pores on 7/8-8/9 Spermathecae however are abnormal, as are other structures in the pre-intestinal portion of the body In these circumstances a quadrithecal battery may also be abnormal

Although male pores are unrecognizable, or perhaps only unrecognized there is a wide median gap in the setal circle of xviii About in region of bc (of xvii or xix) tips of three or four (five on one side of two specimens) setæ are just visible in the epidermis, in a transverse row (smaller specimens) or irregularly arranged and even more closely crowded (larger

worms) This condition is similar to that found on male pore segments of juveniles of penistal species of *Peronyx*. No penial setæ (nor rudiments of male terminalia) have however been found in the coelomic cavity even of, the largest specimen.

Recognition of nephropores is difficult even on carefully relaxed specimens. On contracted worms the pores are usually unrecognizable.

Most of the worms have a more or less dense, epicuticular fauna and flora, so much so that large areas which may involve nearly all of the body, appear white. The primary constituent is a sessile, dichotomously branched, colonial organism (*Rotifera?*) on which there are various sorts of protozoa and protophyta.

P. festivus appears to be distinguished from other South Indian species of the genus by the following combination of characteristics — quadrithecal battery (?) with closely paired pores on 7/8-8/9, nephropores irregularly alternate between dorsum and ventrum, thickened septa posteriorly, gizzard in v, intestinal origin in xvii, presence of hearts in xiii, and absence of nephridial bladders.

Genus *Ramiella* Stephenson, 1921

Six species of this genus, four Indian and two Burmese, have been erected hitherto. Except for one of the Burmese species, all are known only from the original specimens and from accounts that lack information which may well be of taxonomic value. There is also some doubt as to the validity of certain of Stephenson's criteria of specific distinctness, such as number of nephridia per segment and penistal characteristics.

Types of the earlier Burmese species, *parva* Stephenson 1924, are achtellate, perhaps juvenile, and it is possible that characteristics of real specific importance were either unrecognized or not yet recognizable because of immaturity. Of the Indian species two appear at present to be fairly clearly distinguished *heterochaeta* Michaelsen 1921 from Coorg, by the lateral location of spermathecal pores, enlargement of dorsal setæ posteriorly, paired genital markings on 11/12 in bc, location of gizzard in v (possibly incorrect?), and *pallida* (Stephenson, 1920) from the Western Ghats, by loss of the a setæ of viii-ix, location of spermathecal pores on sites of missing follicle apertures, intestinal origin in xvi. *R. pachpaharensis* (Stephenson, 1920) from Rajputana and *bishambari* (Stephenson, 1914) from Saharanpur are doubtful and require consideration in connection with the better known Burmese species, *culturifera*, which has now been found to be common in

the Allahabad region of the Indo-Gangetic plain and thence south to Jubbulpore

Ramiella cultrifera Stephenson, 1931

Material examined —

Allahabad, United Provinces, July, 1 achtellate and 104 clitellate specimens, August, 16 juvenile, 305 achtellate and 260 clitellate specimens, September, 1 juvenile, 310 achtellate and 13 clitellate specimens, October, 3 achtellate specimens November 117 juvenile and achtellate specimens

Naini, Allahabad district, U P., July, 7 clitellate specimens August, 5 clitellate specimens, September, 3 achtellate and 4 clitellate specimens, October, 3 achtellate specimens

Partabgarh, Partabgarh district, U P., September, 28 achtellate and 3 clitellate specimens M Matthur

Madhosingh Junction, Benares State, U P September, 8 achtellate specimens M Matthur

Tunda Falls, Mirzapur district, U P., July, 44 achtellate and 66 clitellate specimens, August, 3 clitellate specimens October, 5 achtellate and 26 clitellate specimens E C Pahari

Mirzapur, Mirzapur district, July, 5 achtellate specimens E C Pahari

Manikpur Junction, Banda district, U P., September, 114 juvenile or achtellate specimens and 6 clitellate specimens M Matthur

Fatehpur, Fatehpur district, U P., September, 44 achtellate and 122 clitellate specimens M Matthur

Nowgong, Chhatarpur State, Bundelskand, Central India, August, 9 clitellate specimens E C Pahari

Chaura, near Nowgong, Central India, August, 3 clitellate specimens E C Pahari

Murbasha Hills, near Nowgong, Central India, August, 1 juvenile, 9 achtellate and 19 clitellate specimens. E C Pahari

Jubbulpore, Central Provinces, October, 6 achtellate and 3 clitellate specimens M Matthur

External characteristics —The first recognizably functional dorsal pore is located as follows on 6/7 (1), 7/8 (13), 8/9 (6), 9/10 (5), 10/11 (1) The clitellum (12/13-17/18) may be saddle-shaped, the epidermis in *aa* transparent, or if annular, the epidermis may be thinner in *aa* than elsewhere

Genital markings are present on some of the specimens from each locality but are small and difficult of recognition. On a series of a hundred

chitellate specimens from Fatehpur genital markings are lacking on 42, present on 58 as follows paired, presetal on viii (3), postsetal on viii (5), presetal on ix (43), postsetal on x (6), postsetal on xi (1), presetal on xvii (1), presetal on xx (1), unpaired and median, postsetal on xix (3), on 19/20 (1) Except on ix where markings are on or near *b* lines, paired markings are usually in *ab*, one of a pair often lacking Occasionally one marking of a pair may be doubled On Allahabad worms markings are on the same locations except that markings are occasionally found on presetal or post-setal portions of vii Paired markings on xx are more common than on Fatehpur worms One of the Partabgarh worms has a pair of well developed genital markings exactly on 10/11, with centres on *b* lines

Internal anatomy—The gizzard is in vi (20, including 6 specimens with genital markings) The intestinal origin is in xiv (20) The typhlosole, from xvii or xviii, is a definite but low ridge, rounded, not lamelliform The last pair of hearts is in xii (20) Lateroparietal trunks are continuous in xiii with the extra-oesophageals One pair of seminal vesicles only, in xii (20)

Remarks—Previous confusion as to segmental location of the gizzard may have been due to failure to recognize and separate septa 6/7 and 7/8

This species can be distinguished at present from *pachpaharensis* only by the "rolled tube" penial setæ This important characteristic was unrecognized by both Stephenson and Michaelson It is therefore possible that the penial setæ of *pachpaharensis*, which must for the present be assumed to be solid, are in reality of the rolled type The figure given by Stephenson (1923, p 400) might well be that of a completely rolled *culturifera* seta in which the margin of overlapping is unrecognizable due to position on the slide There are no significant differences in dimensions or ornamentation of setæ of the two species From the inadequately characterised *bishambari*, *culturifera* is distinguished by the presence of more than one longitudinal rank of nephridia on each side, and by the rolled-tube penial setæ Here again the rolling of penial setæ may have been unrecognized Presence of only one pair of micronephridia per segment in *bishambari* perhaps needs confirmation or questioning In *culturifera* all of the nephridia of one side in a segment may be in contact, and if poorly preserved unrecognizable as discrete organs

Distribution—Hitherto known only from Burma (four localities in central portion) and Christmas Island near Java.

Ramiella nainiana sp. nov*Material examined* —

Naini, Allahabad district, United Provinces, July, 140 juvenile and 37 achitellate specimens. August, 1,009 juvenile, 331 achitellate and 320 clitellate specimens. September, 60 juvenile, 175 achitellate and 503 clitellate specimens. October, 2 achitellate and 412 clitellate specimens. November, 1 achitellate and 171 clitellate specimens.

Rajapur, Allahabad, September, 10 juvenile, 30 achitellate and 28 clitellate specimens

Allahabad, McPherson Lake, September, 2 achitellate and 1 clitellate specimens

Tanda Falls, Mirzapur district, U P, October, 1 achitellate and 8 clitellate specimens E C Pahari

Fatehpur, Fatehpur district, U P, September, 17 juvenile, 22 achitellate and 33 clitellate specimens M Matthur

Lakhaoti, Bulandshar district, U P, "cultivated fields", August, 1 juvenile and 1 achitellate specimens V R Jha

Satna, Rewa State, Central India, October, 2 clitellate specimens E C Pahari

External characteristics — Length 30–65 mm. Diameter $1\frac{1}{2}$ – $3\frac{1}{2}$ mm Number of segments 144–162 (15) Unpigmented, clitellum of live worms brick red but after preservation light yellowish. Prostomium pointed posteriorly, apex reaching onto posterior half of i with a median furrow passing from apex to 1/2, onto ii, or even to 2/3 Rarely the median furrow is lacking and two furrows pass from the nearly pointed apex of the tongue to 1/2 Setæ begin on ii, on xx, ab < cd < bc < aa, dd ca = $\frac{1}{2}$ C; ventral setæ of viii–ix sigmoid The first recognizably functional dorsal pore is located as follows 3/4 (2), 4/5 (101), 5/6 (44), 6/7 (12), 7/8 (10), 8/9 (2), but there are always pore-like markings (possibly functional) on each furrow from 4/5 posteriorly Clitellum annular, reaching anteriorly onto the postsetal or the presetal portion of xiii, rarely to 12/13, and posteriorly onto xvii, occasionally to 17/18 dorsally; intersegmental furrows lacking, dorsal pores occluded, setæ present

Quadrithecal, spermathecal pores minute, on or close to b lines, just in front of the presetal secondary furrows of viii and ix. An annular area slightly peripheral to each pore may be markedly tumescent and with an appearance of a transversely placed tubercle of shortly elliptical outline Female pore median. Apparently there is always but one female pore but a patent aperture is recognizable only on 90 (of 100) specimens Male pores

on the setal arc of xviii, on or slightly lateral to *b* lines, in seminal furrows close to or just on lateral walls. The margin of the pore may be tumescent as an annular lip. Prostatic pores have not been recognized but at each median terminus of a seminal furrow two penial setæ may be protuberant to the exterior independently.

A male field may be clearly demarcated as a transversely placed, dumb-bell-shaped area of marked epidermal thickening on xvii-xix reaching laterally well into *bc*. Usually however the epidermal thickening is lacking or scarcely recognizable in *aa*, the median borders of the longitudinally placed male areas rather indistinct but perhaps just median to the *a* lines. Seminal furrows are deep, rather wide, may have a flat bottom, on the *b* lines, with short terminal portions bent mesially on the setal arcs of xvii and xix.

Genital markings are unpaired and median, reaching laterally to or nearly to *a* lines. Each posterior marking has a single fairly large, greyish translucent, slightly depressed central area clearly demarcated from an opaque, band-like margin. Anterior markings usually have a single, smaller central area but may have two, three or even four all of which are less distinct than on the posterior markings. Preclitellar markings are obviously presetal, occasionally slightly crossing intersegmental furrows. Posterior markings appear to be intersegmental reaching nearly to setal arcs of two segments. On late juvenile specimens anlage of genital markings are first recognizable as greyish translucent areas on the intersegmental furrows. On slightly older specimens the markings appear to be developing more from the anterior than the posterior portions of the neighbouring segments. On later specimens furrows are unrecognizable mesially, apparently ending against the outer edges of marginal bands of the markings about in line with or only slightly behind the level of the anterior margin of central area. Locations of markings are shown below.

Internal anatomy—The gizzard is well developed, in vi (20), intestinal origin in xv just behind 14/15 (20), typhlosole from xvii (14), xviii (5), or xix (1), a simple, fairly high lamella, often reaching to floor of gut, ending abruptly as follows lxxxvii (worm of 116 segments), lxxxviii (of 132 and 146 segments), xcI (of 146 and 148 segments), xcII (of 147 and 150 segments, also in a recently [?] autotomized worm of 103 segments), xcIII (of 151 and 162 segments), xcIV (of 152 segments). In the worm of 103 segments the typhlosole abruptly decreases in height in lxxii, from then on posteriorly gradually fading out. In another worm of but 81 segments the typhlosole ends abruptly in lxxi. The inner wall of the oesophagus in viii-xi is provided with low ridges which are creased or slightly interrupted, in some

specimens apparently vertical, in others apparently longitudinal. In xiii-xiv there are higher, more definitely lamelliform but white ridges which are always longitudinally placed and either not interrupted or only occasionally and more irregularly. In fresh specimens calcareous granules have been found only between ridges of ix-xii. On the floor of the gut midventrally in viii-xii there is a well-developed longitudinal ridge, usually deeply bifid, which may reach upwards halfway to the roof.

Last hearts in xii (20) Lateroparietal trunks when distended with blood may be visible for some distance behind the prostatic segments and in xii¹ are continuous with the extra-oesophageals which can easily be lifted off from the gut. In xiii a small branch from the lateroparietal trunk passes upwards on 13/14 to the vicinity of the dorsal vessel but has not been traced into that vessel.

Excretory system meronephric, nephridia posterior to the prostatic region in three longitudinal ranks on each side, one just lateral to the *d* line, one just lateral to the mid-dorsal line, and one in *ac*. This latter may however be double.

Holandric, testes and male funnels free, seminal vesicles in ix and xii (20). Male deferent ducts of side though in contact are still separate in xviii, passing into the parietes about in line with the prostatic ducts. Prostatic ducts are slender but muscular. Penisetal follicles or muscular strands associated with the follicles pass backwards to the parietes just in front of 19/20 and 21/22, the follicles diverging ectally, one passing into the parietes on the median face of the prostatic duct, the other slightly mesially. The shaft may be nearly straight but usually is curved and variously, length 1.0-1.8 mm., diameter 9-11 μ , except at base which may be considerably thickened. An ectal portion 70-75 μ long is widened and almost membranous, slightly curved at margins and spade-shaped, 20-25 μ wide. The ectal margin is slightly concave. Rarely the concavity may be continued very deeply almost through the membranous portion which then appears to be in two long rods, the shape something like that of a pitch fork without the middle tine. Ornamentation is of triangular teeth which may be few and scattered or more regularly spaced, almost in circles.

The spermathecal duct is abruptly narrowed within the parietes, the major part of the coelomic portion rather columnar and erect in the coelomic cavity, wall muscular, lumen slit-like. A short ental portion which may be slightly widened or narrowed is turned mesially almost at right angles, the ampulla directed upwards or mesially. Often the duct is again turned and ventrally in which case the ampulla is pendent. The rather digitiform

diverticulum passes into the upper face of the first bend of the duct and is directed ventrally along the lateral face of the duct to which it is adherent, never long enough to reach the parietes

Fairly large, acinous ovisacs are present in XIV (20) One or more of the small lobes may contain a brownish material

Glandular material over the genital markings, either on or within the body wall, is unrecognizable, the genital markings apparently only areas of epidermal thickening

Abnormality —(1) The prostomium is cleft horizontally into two approximately equal parts to level of 1/2 (2) The seminal groove of the left side ends with the male pore on XVIII, the posterior prostate lacking (3) The left seminal groove is on XVI-XVIII, with prostates and penial setæ in XVI, and XVIII, the male pore in XVII (4) Seminal grooves are continued on to XX, with an extra pair of prostates and associated penial setæ (5) Left spermathecal pores on VII-VIII, left female pore on XIII, the right on XIV, clitellum on XII-XVI on left side but normal on right side, the left seminal groove on XVI-XVIII The intestinal origin is in XIV on the left side, XV on the right side. Last heart on left side in XI Left testes and male funnels in IX and X, left ovary and oviducal funnel in XII, left ovisac in XIII Left anterior seminal vesicle lacking, posterior vesicles both in XII (6) Spiral metamерism in region of VII-IX

Regeneration —Tail regenerates have been noted at the following levels 40/41, 42/43, 48/49, 49/50, 60/61, 61/62, 64/65, 65/66, 66/67, 69/70 (3), 71/72 (2), 73/74 (2), 75/76, 77/78, 86/87, 88/89, 97/98, 117/118 The regenerate at 40/41 comprises 20 segments, three regenerates have ten or eleven segments, others have three to eight only

Remarks —Small juveniles have ventral setæ of XVII-XIX (apparently sigmoid) in the *a* and *b* lines On slightly larger worms one or both of ventral setæ of XVIII are lacking and the apertures of the *a* follicles of XVII and XIX are slightly lateral to the *a* lines. On still larger juveniles the apertures of *a* and *b* follicles are closely approximated, the apertures of *b* follicles the less displaced

Except as may have been noted to the contrary above, the anatomy is as in *cultifera* (*vide* Gates, 1942, p 122)

R. nainiana is distinguished from all other species of the genus by the location of the spermathecal pores, the unpaired, median postclitellar genital markings, possibly also by the intestinal origin in XV and the sculpturing of the tip of the penial setæ

Genus *Bahlia* gen. nov.

Diagnosis—Biprostatic, male and prostatic pores in seminal grooves on xvii. Bithecal, spermathecal pores on or behind 8/9. Setæ lumbricine, ventral setæ of xvii penial, of ix copulatory. Gizzard in vi; calciferous glands intramural, two pairs, in xi-xii, intestinal origin in xv; typhlosole a simple lamella ending with supra-intestinal glands. Hearts of x-xiii latero-cesophageal, subneural trunk replaced by paired lateroparietal trunks that

Postclitellar genital markings

Location	Naini	Rajapur	Fatehpur
16/17-20/21			1
16/17-22/23		1	
16/17-23/24			1
17/18-22/23			1
17/18-24/25			1
18/19-20/21			
18/19-21/22	1		
18/19-22/23	1	2	
18/19-23/24	1		1
18/19-24/25			1
18/19-25/26			
19/20	2	1	
19/20-20/21	27		2
19/20-21/22	49	5	3
19/20-22/23	144	9	12
19/20-23/24	85	5	7
19/20-24/25	19	.	2
19/20-25/26	2		
19/20-26/27	1	.	1
19/20-27/28	1		
19/20-28/29	1		
19/20-30/31	1		
19/20-31/32			
20/21	30	5	1
20/21-21/22	105	2	2
20/21-22/23	279	8	1
20/21-23/24	231	12	6
20/21-24/25	48	2	5
20/21-25/26	3	1	1
20/21-26/27	2		
20/21-27/28	1	.	
20/21-28/29	1	.	
20/21-30/31	1	.	
21/22	8	.	
21/22-22/23	27	1	
21/22-23/24	55	2	
21/22-24/25	11		
21/22-25/26	4	.	
21/22-26/27	1	.	
22/23	5		
22/23-23/24	12		
22/23-24/25	1		
23/24	1		
23/24-24/25	0	1	

Preclitellar genital markings

Location	Naini	Rajapur	Fatehpur
X-XII	1		
X-XIII			1
XI (duplex)	16 (4)	1	
XI-XII	369 (137) duplex on XI duplex on XII duplex on XI and XII	15 (6)	14 (3)
XI-XIII	6 (2)	1 (1)	(2)
XII	641 (124) duplex	35 (6)	37 (6)
XII-XIII	22 (8)	1	
XIII	1	4	3

"Duplex" indicates two or more central areas, usually two. The figures in parentheses are included in the figure immediately above.

join extra-oesophageals in XIII. Excretory system meronephric. Prostates tubular

Genotype — *Bahlia albida* sp. nov

Distribution — At present known only from a small section of the United Provinces in the southernmost portion of the Indo-Gangetic Plain between Allahabad and Mirzapur.

Remarks — The diagnosis is, of course, only provisional, pending discovery of further species, as intra-generic evolution and variation cannot be predicted.

Relationships, as indicated by the single gizzard the intramural type of calciferous gland, and the presence of supra-intestinal glands, are with *Scolioscolides* Gates, 1937 and *Eutyphlopus* Michaelsen, 1900, but closer to the latter from which it is distinguished as follows by location of spermathecal pores on 8/9, presence of seminal grooves, presence of septa 6/7-7/8, presence of a second pair of calciferous glands in XI, the simple lamelliform typhlosole. Further consideration of relationships is postponed until the excretory system is worked out.

Bahlia albida sp. nov

Material examined.—

Allahabad, United Provinces, July, 3 juvenile and 1 partially clitellate specimens. August, 30 juvenile, 4 achtellate and 5 clitellate specimens,

September, 28 juvenile, 2 achitellate and 2 clitellate specimens, October, 1 juvenile specimen, April, 2 juvenile specimens

Madhosingh Junction, Benares State, U P. September, 1 juvenile specimen.
M Matthur

Mirzapur Mirzapur district, U P, July, 1 clitellate specimen E C Pahari
Tanda Falls, Mirzapur district, U P, July, 1 juvenile specimen E C
Pahari

External characteristics — Length 50–70 mm Diameter 3–4½ mm. Number of segments 155–174 Unpigmented Furrows marking the lateral margins of the prostomial tongue are always continued posteriorly to 1/2, the prostomium presumably to be characterized as tanylobous A transverse furrow may be present at or behind the level of the anterior margin of 1 but presumably is of no more significance than two such furrows present on several worms

Setæ begin on II on which all four couples are usually present From III to VII, VIII or X ventral setæ are larger than the lateral setæ, from VIII or X posteriorly the b setæ also small On X–XIII at least the a setæ emerge from the epidermis close to the anterior intersegmental furrows The a setæ remain large clear to the hind end, the setæ conspicuously protuberant to the exterior and the follicles equally protuberant into the coelomic cavities From the region of XIII–XV, occasionally even anteriorly, the lateral setæ become unrecognizable as do the b setæ behind the genital markings Sites of apertures of setal follicles externally, as well as follicle gaps in the musculature internally, are however recognizable to the hind end In caustic potash preparations of the body wall only a setæ are visible Setal formula, as determined behind the clitellum from sites of follicle apertures would appear to be as follows, cd = or < ab < bc = or < aa, dd ca = ½C Ventral setæ of IX are copulatory and almost always completely retracted into the body wall, when protuberant to the exterior only very slightly so The a setæ, at least of X–XV, have slight ornamentation

The first dorsal pore is on 7/8 (2), 8/9 (20), or 9/10 (11, but usually with a more or less pore-like marking on 8/9) The clitellum which is light yellowish to light brown extends from 12/13 to 19/20 (7) or 20/21 (1) and appears to be saddle-shaped, lacking in aa or bb, intersegmental furrows and dorsal pores lacking, setæ present

Spermathecal pores are rather large, transversely placed slits with centres on or median to the b lines Margins of the pores may be swollen to form a circumferential lip In some specimens with such lips the spermathecal duct can be dissected out from the parietes with the anterior portion

of the *hp* clearly behind 8/9 On other worms the pore appears to be exactly on 8/9 Female pores (paired, 8) are slightly anteromedian to *a setæ* Prostatic and male pores are minute and have not been identified definitely but are almost certainly at anterior and posterior ends of seminal grooves on xvii

A male field is not clearly demarcated, perhaps because of immaturity On ac clitellate, as well as clitellate, Allahabad specimens there is in the region of *ab* on each side a longitudinally placed area of epidermal tumescence, these tumescences usually connected, on clitellate worms, by a shorter area of less marked tumescence to form a transversely placed, rather dumb-bell-shaped marking bearing the seminal grooves The grooves are on or close to the *b* lines, extending from the setal arc of xvii to 17/18, and are crescent-shaped, concave mesially Usually each groove appears to end with the lateral penial seta but occasionally the groove may be extended mesially to include the *a* seta also

Genital markings, transversely placed, in *aa* or *bb*, with clearly marked, white, opaque, raised rims and two, slightly depressed, greyish translucent, central areas, probably are primarily segmental and postsetal but with further growth, and intersegmental furrows unrecognizable, obliterated or perhaps dislocated posteriorly, appear to be intersegmental, located as follows on xviii-xix (9), xviii-xx (4), xviii-xxi (1) In addition three specimens have a more or less symmetrical marking on xvi An asymmetrical marking may have only one central area On the anterior half of ix there is always an area of marked epidermal tumescence, extending well into *bc*, unpaired but in half of the worms with slight incisions of the anterior and posterior margins at the median line and including apertures of follicles of copulatory setæ Greyish translucent spots are lacking on the tumescences which appear to be of the same nature as those associated with copulatory setæ in many species of *Octochætoidea*

Internal anatomy.—All septa are present from 4/5 which is slightly muscular, 5/6-6/7 thickly muscular, 5/6 especially so, 7/8-9/10 muscular

The gizzard is in vi (15), calciferous glands are intramural, like those of *Eutyphæus*, two pairs, in xi-xii (15) In fresh specimens calcareous granules are present between the vertical lamellæ The valve is in xiv, the intestinal origin in xv (15) The typhlosole begins in xix (4), xx (7) or xxi (4), though with a low, short, slightly ridged extension anteriorly, and is of the simple lamelliform type, at first one half to three quarters mm high, ending with the supra-intestinal glands In the first four or five segments the typhlosole is paralleled on each side by a lower but definite secondary typhlosole Supra-intestinal glands are located as follows lxxxii-lxxxvii (1), lxxxiv-xc (1)

Ixxxv-xc (2), Ixxxv-xci (1), Ixxxvi-xci (2), Ixxxvi-xci (4), including the Mirzapur specimen)

The dorsal blood vessel (single) is continued on to the pharyngeal bulb and after giving off a fairly conspicuous branch which passes upwards above the brain, passes underneath the brain where it bifurcates. A supra-oesophageal trunk is recognizable only in xi-xiii and may be double in any one, two or all three of the segments, but with the two portions united to pass through a septum. Extra-oesophageals unite mesially on the ventral face of the gut in ix or x. Lateroparietal trunks are gorged in xiv-xx and are lateral to the prostatic ducts, passing on to the ventral face of the gut, apparently to the extra-oesophageals, in xiii. In favourable specimens these parietal trunks may be recognizable in part or *in toto* to the hind end. Hearts of xi-xiii are latero-oesophageal. Hearts of x are smaller and appear to open only into the dorsal trunk, bifurcations passing to the gut definitely recognizable only in one worm. Lateral loops are present in v-ix.

Metandric, one pair of large digitate testes and of male funnels (without iridescence) in xi. Seminal vesicles in xii push 12/13 back into contact with 13/14. The prostatic duct is less than one mm long and may be looped. Vasa deferentia in xvii are lateral to the prostatic ducts and just in front of 17/18 pass downwards into the parietes without enlargement or turning mesially.

The spermathecal duct which is shorter than the ampulla is slightly spindle shaped. The diverticulum which passes to the lateral face of the wider middle portion is usually almost spheroidal and with almost no stalk but may be flattened and rather disc-like. Spermatozoa are arranged in numerous, small, spindle-shaped masses at the periphery (2 specimens possibly postsexual).

Penial setæ are in two distinct follicles separated ectally by strands of longitudinal musculature, the lateral follicle passing into the parietes on the median face of the prostatic duct. The setal shaft is curved into a crescent or parenthesis shape. An ectal portion is considerably thinner, almost membranous, but not narrowed or widened from side to side, the lateral margins curved so as to produce a rather scoop-shape, the terminal margin slightly indented. Ornamentation is of 20-30 complete or irregularly interrupted circles of teeth varying in size, the larger almost triangular, the smaller more thorn or spine-like. The ornamentation may be continued on the scoop nearly to the ectal end. Length 1 mm. Diameter in region of greatest thickness ca 40 μ .

Follicles of the copulatory setæ (*a* and *b* of ix) are larger than those of the *a* setæ of neighbouring segments and are long enough to project conspicuously into the coelomic cavities but are bent over laterally and bound to the parietes Copulatory setæ are very similar in shape of the slightly clawed tip, and the ornamentation of numerous depressions (shallow ectally but deepened entally) to the copulatory setæ of several species of *Octochetoides* Length 0.65-0.7 mm Diameter in region of greatest thickness 23-35 μ

No glandular material is recognizable on the parietes over sites of genital markings which appear to be areas of epidermal modification only

Parasites—Small nematodes and gregarine protozoa rather like *Aikinetocystis singularis* are present in the coelomic cavities

Regeneration—Several juvenile specimens appear to have been regenerating short tail portions Another juvenile had lost its head behind xvii At the anterior end there is a flat, circular area of regeneration tissue with an imperforate brownish spot centrally Setæ of first two segments are wholly lacking

Remarks.—The Mirzapur worm differs from clitellate Allahabad specimens as follows Clitellum annular, except on xviii-xix where it is lacking in *bb*, dark reddish brown Male field clearly demarcated, extending from 17/18 forwards nearly to 15'16, just including the *a* setæ of xvi, posterior margin straight, anterior margin convex Seminal groove tumescences unrecognizable, genital markings lacking but in anterior portion of male field, just median to a line on each side a longitudinally placed area of greyish translucence Copulatory tumescence on ix crosses whole length of segment Dorsal pores lacking anterior to clitellum Spermathecal pores exactly intersegmental. Spermathecal duct rather barrel-shaped These differences, if constant on Mirzapur worms, may be sufficient to warrant recognition of a second species

The smallest juvenile found is at once recognizable by the conspicuously protuberant, enlarged *a* setæ and absence of other setæ posteriorly On this worm the ventral setæ of xvii are small and only very slightly displaced from the *a* and *b* lines.

April specimens were hibernating Each was coiled tightly into a ball within a cell about seven by ten mm, some 15 to 20 mm below the surface of the earth After clods had been broken open, exposing the animals to noon-heat of a mid-April day, no movement was observed and no circulation of blood was recognizable though the dorsal blood vessel was clearly visible When dropped into water the worm at once uncoiled but thereafter remained

motionless though occasional pulsations of the dorsal vessel became visible
After fixation cloacal cavities were found to be filled with a very viscous
fluid Size ca 60 > 3 mm

Genus Calebiella gen nov

Diagnosis — Quadriprostatic prostatic pores on xvii and xix, male pores on xviii, in seminal grooves Quadrithecal, spermathecal pores on viii and ix Setæ lumbricine Gizzard in vi Calciferous glands extra-mural, four pairs, in x-xiii Subneural replaced by paired lateroparietal trunks Hearts of x-xiii latero-oesophageal Excretory system meronephric Prostates tubular

Genotype — *Calebiella parva* sp nov

Distribution — Known at present only from the type locality of the type species, Partabgarh, United Provinces

Remarks — Diagnosis is of course tentative, pending discovery of further species The genus resembles *Ramuella* in the location of the single gizzard and characteristics of the spermathecae but is clearly distinguished by the well-developed calciferous glands Copulatory setæ with associated epidermal tumescences and the bifid typhlosole are as in species of *Octochætoides* but similar copulatory setæ are also found in species of *Pellogaster*, *Iennogaster* and *Eudichogaster* The calciferous glands and the excretory system distinguish the genus from *Octochætoides*

Calebiella parva sp nov

Material examined —

Partabgarh, Partabgarh district, United Provinces, September, 2 acilitellate and 2 clitellate specimens M Mathur

External characteristics — Length 65 mm Diameter (through swollen clitellum) 2½ mm Number of segments 140-143 (2) Unpigmented Prostomium combined pro- and epilobous (1) or possibly prolobous (3) Setæ begin on ii on which all are present, a and b of xvii and xix penial, of ix copulatory, of xviii lacking, on xxv, ab ca - cd < bc < aa, dd ca = ½ C First dorsal pore on 9/10 (2), 10/11 (2) Clitellum annular, light yellowish, from 12/13 to or nearly to 17/18 dorsally, lacking ventrally on xvii, intersegmental furrows lacking, setæ present, dorsal pores all (1) or in part open (1)

Quadrithecal, spermathecal pores minute and superficial, on b lines, on presetal secondary annuli of viii and ix but nearer to presetal secondary than to intersegmental furrows. Female pores unrecognizable, probably

paired Prostatic pores not identified Male pores minute, on setal arc of xviii and on lateral walls of seminal furrows

Male field not marked off Seminal furrows in *ab* close to *b* lines, bent mesially on setal arcs of xvii and xix, slightly concave laterally on xviii, margins slightly tumescent

Genital markings possibly represented by paired, transversely placed, rather indefinite, presetal areas of epidermal translucence reaching mesially nearly to the mid-ventral line, and laterally, on xix to the seminal furrows, on xvii to slightly beyond the male pore lines On ix there is a large unpaired area of marked epidermal tumescence reaching laterally nearly to the *c* lines, anteriorly to the presetal secondary furrow and posteriorly except at the mid-ventral line to 9/10 (1) The other three specimens appear to indicate that paired tumescences first appearing in *ab* on a postsetal portion of the segment gradually increase in size until mid-ventral union occurs There are no areas of translucence within the tumescent region which accordingly is similar to that associated with copulatory setæ in species of *Octochaetoides*

Internal anatomy—Septa 4/5-6/7 thickly muscular, the muscularity decreasing posteriorly, 7/8-13/14 with muscular fibres but translucent.

Gizzard, large and strong, in vi(4) Oesophagus slender Calciferous glands four pairs, in x-xiii (4), each gland rather reniform, apparently attached without stalk by hilus to gut at or slightly below mid-lateral line, each gland more or less deeply constricted into two nearly equisized lobes Glands of a segment are in contact well below the ventral surface of the gut Oesophagus narrowed in xiii-xiv, almost valvular, but real valve apparently in region of insertion of 14/15, the intestinal origin in xv just behind 14/15 (4) Typhlosole begins in xvii, reaching full height in xviii or xix, bifid ventrally from beginning, the bifurcations perpendicular or recurved so that a cross-section is of an inverted T- or Y-shape The bifurcations gradually disappear posteriorly, the remainder of the typhlosole simply lamelliform, ending abruptly in xcix (worm of 140 segments) or ciii (of 143 segments) In the last three typhlosolar segments there appear to be three pairs of supra-intestinal glands, dark red These are however hollow rather than solid and may be merely artefacts due to some unusual contraction of the dorsal wall of the gut

Dorsal blood vessel (single) passes on to the pharyngeal bulb anteriorly Supraoesophageal recognizable only in x-xiii Extra-oesophageals free from gut through ix, unrecognizable posteriorly Lateroparietal trunks recognizable for some distance behind the prostatic region, passing upwards in xiii, connection with other trunks unrecognizable No subneural Hearts of

x-xiii apparently are latero-oesophageal, but bifurcations to dorsal trunk are very slender and white (last pair in xiii, 4) Large lateral commissures are present in v-ix

Excretory system meronephric Anterior to 4/5 there is a pair of large clusters of pharyngeal (?) nephridia From x posteriorly nephridia are in three longitudinal ranks, on each side, of tubules transversely placed on the parietes Posteriorly the median nephridium on each side is enlarged and apparently provided with a preseptal funnel

Holandric, testes and male funnels free, seminal vesicles small, acinous, in ix and xii (4) Prostatic ducts short, straight, slender Male deferent ducts pass under the glandular mass of xvii and just lateral to the prostatic ducts, disappearing from sight in xviii

Penisetal follicles long, those of xvii attached to parietes in xxiii, of xix in xxiv The setal shaft may be nearly straight, variously curved or bent Length ca 3 mm., diameter 10 μ An ectal portion which may be 50-80 μ long and 30 μ wide, is very thin, almost membranous, curved into a sort of scoop-shape, but with ectal margin rounded

Spermathecal duct longer than ampulla, slender, circular in cross-section, a very short ental portion bent over sharply, (and mesially ?) almost at right angles to the rest of the duct Diverticulum slightly more than half the length of duct, passing into dorsal face of the bent portion, very slightly narrowed dorsally. In the ventral portion of the diverticulum, there appear to be a number of very small chambers

Follicles of ventral setæ of ix are large enough to project conspicuously into the coelomic cavity but are bound to the parietes laterally Setal shaft nearly straight to slightly arced Length 0.4-0.45 mm., maximum diameter 12-16 μ The tip, ca 30 μ long, is clawed as in species of *Octochatoides* and may be hollowed out considerably The ornamentation may extend nearly half-way down the shaft and is of longitudinally placed gouges, deepened entally, the floor smooth ectally but entally roughened and with a granular appearance. At any particular level along the shaft the maximum number of gouges visible at one time is three, one at the centre and two at the margins.

Glandular (?) material over sites of genital markings is flattened out on the parietes but may easily be lifted off

Remarks — Neither of the clitellate specimens is sexual, no spermatozoal iridescence on male funnels or in spermathecae. This may explain the indistinctness of the supposed genital markings which were not observed until finding of the glandular (?) material internally The junction of the

diverticular with the duct lumen has not been found. Two at least of the specimens appear to be complete, unautotomized. If so, the typhlosole extends further back than usual. As the posterior segments are short the portion of the body without typhlosole is only 8-10 mm long.

Supposed funnels of median nephridia posteriorly are small and very delicate,—attempts to mount them for microscopic examination failed.

Genus *Pellogaster* Gates, 1939

Pellogaster isabellae sp. nov.

Material examined —

Naini, Allahabad district, United Provinces, July, 20 juvenile and 10 acilitellate specimens; August, 200 juvenile, 201 acilitellate and 25 clitellate specimens; September, 22 juvenile, 10 acilitellate and 4 clitellate specimens; October, 4 acilitellate specimens.

Fatehpur, Fatehpur district, U.P., September, 8 juvenile and 1 acilitellate specimen; M. Matthur

External characteristics — Length 40-70 mm. Diameter (through swollen clitellum) 2 mm. Number of segments 141-153 (3 specimens). Prostomium epilobous, tongue short, open (44), apparently prolobous (3), or combined pro- and epilobous (3). Setæ begin on II on which all are probably present though retracted so that some or all are usually unrecognizable, all ventral setæ of VIII-IX present, on XXV, $ab < cd < bc < cd$, $dd \approx \frac{1}{2} C$. The first recognizably functional dorsal pore is located as follows on 7'8 (1), 8/9 (2), 9/10 (45, but with a pore-like marking on 8/9—41), 10/11 (1), 11/12 (1). Possibly the first pore is almost always on 8/9. Clitellum from 12/13 to or nearly to 17/18 dorsally, lacking ventrally on XVII.

Spermathecal pores minute, superficial, in *ab*, usually nearer to *b* lines, on or just in front of setal arc of IX, slightly in front of setal arc of VIII, or nearer to or actually on the presetal secondary furrow, rarely (1) in front of the furrow. Female pores unrecognizable as patent apertures though sites (paired) are clearly marked on most clitellate specimens. One worm apparently has a single, median pore. Male pores minute, on setal arc of XVIII and on lateral walls of seminal grooves. Prostatic pores not identified.

Male field not definitely marked off. Seminal grooves nearly straight, in or close to *ab*, shortly bent mesially on setal arcs of XVII and XIX, walls slightly tumescent.

Genital markings small, circular or shortly elliptical (and then transversely placed), except on XII with fairly wide, opaque, marginal areas clearly

marked off from greyish translucent, slightly depressed, circular, central areas. Markings of XII, in a transverse row, included within a sharply demarcated, transversely placed band of marked epidermal thickening, presetal or just including the ventral setæ within the posterior margin, reaching laterally with rounded ends into *bc*. Each clitellate and achatellate specimen has two or more markings on XII. On fifty specimens markings are located as follows: paired, in *aa*, on XII, (49), in *ab*, on or lateral to *b* lines (41), presetal in *aa*, on XVI (4) on XVII (8) on XX (1), postsetal, with centres on *b* lines, on XVI (1), in *ab*, on XIX (26), unpaired median, presetal on XX (1), on setal arc of XXI (1).

Internal anatomy.—The oesophagus is markedly constricted in the region of insertions of 9/10-12/13 and moniliform in IX-XII, dark red segmentally in X-XII, the ventral face smoothly convex without indications of calciferous pockets. On the floor of the gut in X-XII is a large, median, deeply bifid ridge which may reach up into contact with the roof. Usually the bifurcations are in contact with the lateral walls of the gut thus closing off on each side a small ventral chamber which has on its floor midsegmentally a few low lamellæ. In XII-XIII the oesophagus is slenderer white, the inner wall longitudinally ridged, almost valvular in appearance. The real valve however is located in the region of insertion of 14/15. Intestinal origin in XV just behind 14/15 (20). The typhlosole which begins gradually or abruptly in XVII-XIX (if gradually reaching full height in XVIII-XX) is a simple lamella which may be one mm. high with no marked decrease in height posteriorly, ending abruptly in LXXX (worm of 141 segments), LXXXIII (153 segments), or LXXXV (150 segments).

Extra-oesophageal trunks disappear into the ventral face of the gut in the region of 9/10, apparently continued posteriorly within the ridge on the floor of the gut, emerging again on to the ventral face in the region of 12/13 and recognizable back to 13, 14. Neither lateroparietal nor subneural trunks have been recognizable (20). Last pair of hearts in XII (20).

Holandric, testes and male funnels free, seminal vesicles acinous, in XI and XII (20). Prostatic ducts slender, slightly looped, muscular sheen unrecognizable. Ventral ends of penisetal follicles (or muscular strands therefrom) of XVII attached to parietes in XIX, of XIX attached in XXI. Penial setæ are 2.0-2.3 mm. long, 6-8 μ thick, nearly straight or variously curved. The tip is bluntly rounded (worn?), truncate (broken?), sharply pointed, or continued into a short, hair-like spine. Ornamentation sparse, indistinct, of a few small, triangular teeth occasionally recognizable only by incisions at margins of the shaft.

Spermathecal duct slender, circular in cross-section, nearly as long as, to definitely longer than the ampulla. The diverticulum is digitiform, directed ventrally along the duct (on the lateral face?) to which it is adherent, opening into the ental end of the duct just below the ampulla. The digitiform characterization applies only to diverticula filled with spermatozoa. When empty an elongately ellipsoidal seminal chamber with simple lumen is distinguishable from a shorter, only very slightly narrower but thicker-walled stalk. Occasionally the proximal portion of the stalk is distended or constricted so as to produce something of an appearance, at first glance, of a second and smaller diverticulum. Within the parietes the duct is abruptly narrowed. This portion is very easily pulled out from the parietes leaving in the epidermis a clearly recognizable opening, thus enabling certain determination of position of the spermathecal pore when the latter is not definitely recognizable.

Follicles of the ventral setæ of VIII-IX are always (20) protuberant into coelomic cavities but are bent over laterally and attached to the parietes. Copulatory setæ are 0.31-0.36 mm long, $\approx 10\mu$ thick. The tip is slightly clawed, a portion ental to the claw ornamented with rather closely crowded, slight gouges. The shape of the tip and the ornamentation is very similar to that of copulatory setæ of several species of *Octochaetoides*.

Glands over sites of genital markings either on or within the parietes, have not been found.

Regeneration — Three worms have, at unknown levels behind 19/20, regenerates that look like heteromorphic tails.

Remarks — The anatomy, except as has been indicated to the contrary above, is as defined for the genus, or as in *bengalensis* (Michaelson, 1910), the type species.

Preservation is unsatisfactory for study of the excretory system. As in *bengalensis* there is obviously a longitudinal rank of nephridia just lateral to a line on each side from IX or X posteriorly. At least one other rank is present, in ac. In that area in the prostatic region there appear to be three or four small nephridia on each side per segment, but posteriorly only one nephridium on each side is definitely recognizable, and this appears to be, like the lateral nephridium, a rather large, perhaps saccular organ transversely placed on the parietes. The excretory system of *Pellogaster*, according to Bahl (1942, p. 446), "closely resembles" that of *Eutyphaeus*. As a rather general statement that may of course be correct but perhaps requires confirmation or some qualification. The transversely placed nephridia, especially in the lateral ranks behind the prostatic region, look more like the

nephridia of *Ramiella* which Bahl appears to consider saccular, than the Y-shaped, parietal nephridia of *Eutyphaeus*. Of the latter sort of nephridia not a trace is recognizable in the present specimens, though this may perhaps be due to faulty preservation.

P. isabella is clearly distinguished from all of the forms hitherto described by the presence of the *a* setæ of viii and ix, the copulatory setæ of viii-ix with clawed tips and ornamentation like that in certain species of *Octochætoides*, the constant presence of a transverse row of presetal genital markings on xii, intestinal origin in xv, the long capilliform penial setæ, and perhaps also by several characteristics of minor importance such as shape of prostomium and location of first dorsal pore. The species appears to be more primitive than the forms previously described in the absence of calciferous pockets on the ventral face of the gut and restriction of the calciferous region to x-xii.

Genus *Lennogaster* Gates, 1939

Lennogaster elongatus sp. nov.

Material examined—

Nowgong ("Grain Market, Gola Bazaar"), Chhattarpur State, Bundelskand, Central India, August, 1 achatellate specimen E C Pahari

External characteristus—Length 120 mm Diamater 3½ mm Number of segments ca 185 Unpigmented Prostomium proepilobous, very slightly indenting 1 but with a deep median furrow from posterior apex of tongue to 1/2 Setæ begin on ii on which all are present, on xxx, *ab* < *cd* < *bc* < *aa*, *dd* ca = ½ C First dorsal pore on 12'13

Spermathecal pores minute and superficial, on viii and ix, about on *a* lines, on or just behind the presetal secondary furrows, each pore at centre of a nearly circular slight turnescence Female pores paired Male and prostatic pores unrecognizable

Male field fairly clearly demarcated, almost rectangular, longitudinally placed, extending from 16/17 nearly to setal arc of xxi Seminal furrows nearly straight, in *ab*, extending from the setal arc of xvii to 20/21 The anterior ends are bent mesially on the setal arc of xvii A short transversely placed furrow on each side of the setal arc of xix and opening laterally into the seminal furrow, apparently corresponds to the anterior portion of the furrow. Ventral setæ of xx as well as of xxi are present

Internal Anatomy—The anterior septa are thickened and 8/9 is thickly muscular The typhlosole begins in xx-xxi and is a fairly high, simple

lamella, ending abruptly in cxvi, apparently without posterior enlargement or lateral lamellæ

Holandric, testes and male funnels free, funnels with slight spermatozoal iridescence, seminal vesicles in ix and xii Prostates fairly well developed, zigzag-looped, duct slender but muscular Male deferent ducts pass into parietes midsegmentally in xviii, uniting within the body wall Penial setæ 0.47-0.56 mm long, 10-12 μ thick, shaft nearly straight or very slightly arched, tip chisel-shaped, sharply pointed or ending in a short sharp spine Ornamentation sparse, of a few small, rather triangular teeth Follicles of ventral setæ of vii-ix are not enlarged A reserve setæ from an *a* follicle of vii is definitely sigmoid

Spermathecal duct about as long as wider ampulla, from which it is clearly marked off, slightly and very gradually narrowed ectally The diverticulum is less than half the length of the duct, shortly digitiform, apparently ventrally directed and opening into ental end of the duct

Remarks — Except as noted above the anatomy is as in *falcifer* from which the present species is distinguished as follows by the definitely greater size, much longer typhlosole, absence of modifications of a posterior portion of the typhlosole, smaller penial setæ, ventrally directed spermathecal diverticulum and the normal sigmoid shape of a setæ of vii If the type is normal, the posterior extension of the seminal furrows to 20/21 and of the male field onto xx will distinguish *elongatus* from all other species of the genus There is however a possibility that this posterior extension is abnormal though similar posterior extension (on abnormal specimens) is usually associated with presence of extra prostates which are lacking here

Lennogaster falcifer (Stephenson), 1920

Material examined —

Jubbulpore, Central Provinces, October, 5 acitellate and 13 clitellate specimens M Matthur

External characteristics — Length 40-70 mm Diameter (through swollen clitellum) 2-3 mm Number of segments 142-151 (4) Unpigmented, clitellum yellowish to brownish Prostomium proepilobous, indenting it only slightly but with a deep median furrow from posterior apex of tongue to 1/2 Setæ on xxv, usually, $ab < cd < bc < aa$, $dd = \frac{1}{2} C$, but cd , bc and aa may be nearly = First dorsal pore on 12/13 (12, but with a somewhat pore-like marking on 11/12-4) Clitellum annular, from 12/13 to 16/17, onto xvii or to 17/18 dorsally, intersegmental furrows and dorsal pores lacking, setæ present

Spermathecal pores minute and superficial, each at centre of a small, sharply demarcated, circular area which is often raised and papilliform, pores on or just lateral to *a* lines, on or just in front of presetal secondary furrows of viii and ix. Female pores paired (5). Male pores minute, on setal of arc of xviii on roof of seminal furrows. Prostatic pores unrecognizable.

Male field sharply demarcated, shortly elliptical, transversely placed, reaching to 16/17 and 19/20 and laterally nearly to *c* lines, a narrow rim especially protuberant, clearly marked off both peripherally and centrally. Seminal furrows concave mesially, crescentic to U-shaped. The region of *aa* just behind the rim on xvii and just in front on xix, usually greyish translucent, often depressed.

A small, rather indefinite area surrounding the aperture of each *a* follicle of vii is usually slightly tumescent, occasionally distinctly demarcated and then circular. On two specimens, the circular areas are especially protuberant and like small tubercles, both included in a single clearly marked transversely placed area of epidermal thickening reaching to 6/7-7/8 and well into *bc*. Five specimens apparently have paired genital markings in *aa* on xviii and one has a single median area of tumescence in *aa* on 20/21.

Internal Anatomy -- Septa 5/6-6/7 membranous, transparent, 7/8 translucent, 8/9-10/11 muscular. Gizzards in v-vi (13). The typhlosole begins in xix (6) or xx (7) and is a simple, high lamella, ending abruptly in ix (specimen with 114 segments of which the last few may be a tail regenerate), lxxx (of 142 segments, and also in an autotomized specimen of 93 segments), lxxxi (of 143 segments), lxxxii (of 145 segments, and also in an autotomized specimen of 106 segments) lxxxiii (of 151 segments). Except in the last two segments a posterior portion has lateral, diagonally placed lamellæ but in addition the main lamella appears to be considerably thickened marked off internally into two portions in each segment, and with ventrally a longitudinal blood vessel or sinus close to the margin.

Fxtia-cesophageals disappear into the ventral face of the gut in x, apparently continued posteriorly in the lateral ridges that cover over apertures into calciferous glands. Lateroparietal trunks pass into xiii but cannot be traced to connections with other vessels.

Nephridia, behind the prostatic region, transversely placed on the parietes, in a transverse row of at least four on each side in each segment.

Holandric, testes and male funnels free (13), seminal vesicles acinous, in ix and xii. Prostates are fairly large and with several loops. Male

deferent ducts of a side unite as they pass into the parietes in xviii. Penial setæ 0.75-0.96 mm long, maximum thickness 12-16 μ , shaft nearly straight, or with an ectal portion in a single, slight curve, only rarely sickle-shaped as figured by Stephenson. The tip is narrowed and may be rather chisel-shaped but usually is softened at the ectal margin, almost membranous, or ending in a short hair, or even bifid, usually crumpled. Ornamentation may be almost unrecognizable, indicated only by slight marginal incisions as in Stephenson's figure (1923, p. 413, Fig. 214) or of numerous, fairly large, triangular teeth. A short ental portion of the shaft but of variable length may be tubular and with tissue passing into the hollow.

Follicles of the ventral setæ of viii and ix are concealed within the parietes. The follicles of the *a* setæ of vii however are conspicuously protuberant into the coelomic cavity, long enough to reach over laterally just beyond the *b* lines, triangular, apex dorsally, lateromesially flattened. Setæ are 0.44-0.48 mm long, 14-16 μ thick at middle, 20 μ or more entally without nodulus, major portion of shaft nearly straight, a short ental portion curved like handle of a cane, tip rounded, unornamented. One worm has no spermatheca in viii on the left side and the left *a* follicle is not enlarged nor protuberant into the coelomic cavity.

The spermathecal duct is about as long as the much wider, clearly demarcated ampulla which may be shortly ellipsoidal, thickly ovoidal, heart-shaped, or collapsed and crumpled. The diverticulum is shorter than the ampulla rather digitiform, usually without recognizable narrowing near the duct, passing out from ental end of duct at a right angle but then usually curved upwards along one side of the ampulla. There is here, accordingly, none of the difficulty of determination of limits of duct and ampulla, as in *chittagongensis* and certain other species.

Remarks - These specimens have a fairly large fauna and flora attached to the cuticle which may be responsible for the rather poor condition. The epidermis in particular is roughened, wrinkled or creviced, especially so on the chitellar region and on vii where the markings associated with copulatory setæ are recognizable only with difficulty or in some cases perhaps quite unrecognizable.

Except as has been noted to the contrary above the anatomy is as in *chittagongensis* and other species of the genus.

L. falcifer has been known hitherto only from the original account of the types which probably were juvenile as prostates were "scarcely developed". Locations of reproductive apertures were not determined, gizzards were referred to vi-vii as septa anterior to 6/7 were unrecognizable.

Present specimens differ in spermathecal conformations and quite definitely in dimensions of the penial setæ. Such differences are to be expected in view of immaturity of the types. As some of the original specimens were from Jubbulpore there appears to be no reason for questioning the identification.

Lennogaster pusillus (Stephenson) 1920

Material examined —

Naini Allahabad district, United Provinces, July, 4 chitellate specimens

August, 23 achitellate and 131 chitellate specimens, September, 1 achitellate and 70 chitellate specimens, October, 2 chitellate specimens

Allahabad, "earth around roots of pointed plants", July, 4 chitellate specimens

Tunda Falls, Mirzapur district, U P, August, 3 juvenile 2 achitellate and 13 chitellate specimens E C Pahari

Manikpur Junction, Banda district, U P, September, 1 achitellate specimen M Matthur

Nowgong, Chhatarpur State, Bundelskand, Central India August, 1 achitellate and 48 chitellate specimens E C Pahari

Nowgong, Gola Bazaar, August 1 achitellate and 92 chitellate specimens E C Pahari

Chaubara, west of Nowgong, August, 19 chitellate specimens E C Pahari

Jubbulpore, Central Provinces, October, 23 chitellate specimens M Matthur, Bombay, August 2 chitellate specimens K N Bahl

External Characteristics — Length 20–60 mm Diameter 1–2½ mm Unpigmented, body wall of live-worm transparent, chitellum recognizable only by a white opacity, chitellum of preserved worms greyish, light yellow, orange or brownish Prostomium proepilobous but front sharply pointed tongue deeply indenting in a median furrow is continued to 1/2 Setæ begin on II on which all are present, intersetal ratios variable, on XXV, usually $ab < cd < bc < aa$, $dd ca = \frac{1}{2} C$, but cd , bc and aa may be about equal First dorsal pore almost always on 11·12, on 12/13 one specimen

Spermathecal pores on or just in front of presetal secondary furrows of VIII and IX, on or immediately lateral to a lines Female pores, paired, anteromedian to a setæ to which they are nearer than to each other Male and prostatic pores unrecognizable, but probably as in *L. chittagongensis* (Stephenson), 1917, as are the seminal grooves

Each seminal groove is on a small, elliptical area of marked epidermal thickening, sharply demarcated by a completely circumferential furrow, the

porophores diagonally placed (convergent anteriorly) or transversely placed A presetal portion of xvii and of xviii, in *aa* or *bb* (on xviii) is usually greyish translucent, often depressed, occasionally sharply demarcated and then with something of an appearance of a transversely elliptical genital marking. On some specimens the areas are raised, even more sharply demarcated but opaque with no trace of translucent areas The posterior margin of the clitellum is markedly and regularly concave

Internal anatomy —Gizzards are in v-vi (25), intestinal origin in xv (25), typhlosolar origin in xvii (6) or xviii (19), the typhlosole a simple lamella, often reaching floor of gut enlarged at the hind end and provided there with lateral lamellæ, often simple again in the last two segments, ending abruptly in lxx (of 118 segments), lxxv (133 segments), or lxxvi (Bombay specimen of 130 segments, autotomized Allahabad specimen of 109 segments)

Extra-oesophageal trunks apparently pass into the anterior calciferous glands. Lateroparietal trunks have been traced from the hind end of the body into xiii where they turn upwards and after passing through 12/13 disappear from sight into the posterior calciferous glands Last hearts in xii (25)

Nephridia in xiv-xvi may be 16 per segment and are more or less erect not flattened out and adherent to the parietes Behind the prostatic region there are four longitudinal ranks on each side of transversely placed tubules on the parietes

Proandric, testes and male funnels (of x) in paired testes sacs (25), seminal vesicles lacking Each specimen (25) has a pair of male funnels free in xi Prostatic ducts ca $\frac{1}{2}$ mm long, slender, straight, with slight muscular sheen Male deferent ducts pass into the parietes posteriorly in xvii, behind the prostatic ducts

Penial setæ 0.6-0.64 mm long, ca 5μ thick, shaft straight, slightly curved, occasionally in part rather sinuous A very short tip portion is almost membranous, very slightly widened, curved, rarely with rounded ectal margin, usually straight, jagged, concave or more or less deeply indented in which case the shaft appears to end in two, short fine spines Ornamentation sparse, of rather small triangular teeth, usually recognizable only by marginal incisions

A short ectal portion of the spermathecal axis, slightly protuberant into the coelomic cavity is slender, circular in cross-section and with a smooth surface. Entally this portion bends over mesially almost at a right angle At the angle there is protuberant laterally a small rounded body presumably

a diverticulum. The major portion of remainder of the axis is somewhat widened, irregularly constricted. Usually a much shorter terminal portion is still further widened, shortly ellipsoidal to ovoidal and ampulliform in appearance.

Follicles of *a* and *b* setæ of VIII are equisized, no larger than those of other segments, not conspicuously protuberant into coelomic cavities. The *a* and *b* setæ of VIII are sigmoid and unornamented.

Regeneration—Two specimens have short tail regenerates at 50/51 and 58/59, 2 mm long, comprising 15 and 11 segments and a growth zone.

Remarks—Except as may be indicated to the contrary above, the anatomy is as in *chittagongensis* (*vide* Gates, 1939, p 192).

Only four of the dissected specimens have any iridescence on male funnels of X and then only slight. No spermatozoal iridescence has been found in any of the spermathecae. The latter are refractory to clearing agents and a satisfactory characterization of the portion from the diverticular junction entally is still needed. Ovisacs may contain a few ova, brownish granular debris, both or neither.

The smallest juvenile that has been identified has seminal grooves but no trace of the porophores. Slightly larger juveniles have the margins of the grooves slightly tumescent.

L. pusillus is known only from the original description of a single specimen from Saugor Central Provinces. Locations of spermathecal and female pores were not determined "slits", probably seminal furrows, were characterized as "transverse" prostatic pores, locations of gizzards were not certain, and the tip of the penial seta was described as "flattened and slightly expanded". No mention is made of copulatory setæ. The penisetal tip of specimens described above appears to be more like that figured for *barkudensis* (Stephenson, 1923, p 409, Fig 211) than that figured for *pusillus* (*Ibid.*, p 418, Fig 218).

Reasons for identification as *pusillus* rather than *barkudensis* are as follows. All of the present localities, and in particular Jubbulpore, are much nearer the type locality of *pusillus* than that of *barkudensis* (Barkuda Island, Chilka Lake, on the Orissa Coast). *L. barkudensis* is distinguishable from *pusillus* only by a very slight difference in shape of the penisetal tip which may be unimportant even if real. Experience with one seta from an Allahabad worm seems to indicate that the difference is fictitious. The tip which at first seemed to be spearhead-shaped appeared so only because of the oblique angle at which it was held. When the shaft was rolled over

slightly the characteristic shape, as described above, was at once recognizable (Difference in shape of the "prostatic pores", really seminal furrows, is probably of no importance. When the porophore is transversely placed, the groove at first appears to be transverse, correct characterization usually recognizable only on careful examination with the highest magnification.)

L. pusillus is distinguished from *chittagongensis* by absence of copulatory modifications on *a* setæ of xviii and possibly by slight differences in location of the spermathecal pores and in the development of the male field on xvii–xviii.

Lennogaster trichochætus (Stephenson), 1920

Material examined —

Bombay, August 2 clitellate specimens Prof K N Bahl

External Characteristics —Unpigmented Prostomium proepilobous with a median furrow from apex of tongue to 1/2 or onto ii. First dorsal pore on 12/13 (2). Female pores paired, slightly anteromedian to *a* setæ. Margins of follicle apertures of copulatory setæ slightly tumescent and resembling small tubercles. Terminal portions of seminal furrows are transversely placed on setal arcs, reaching to *a* lines, concave posteriorly on both xvii and xix. Male field raised, definitely demarcated.

Internal anatomy —Intestinal origin in xv (2). Typhlosole begins rather gradually in xix (2) and is a simple lamella, ca ½ mm high, ending abruptly in lxxx (1), in lxxi–lxxviii with 12 diagonal ridges on each side, the posterior ends dorsally, the anterior ends at or near the ventral margin of the typhlosole. Paired, shortly stalked bodies, possibly lymph glands, are attached to the posterior faces of the septa some distance lateral to the dorsal vessel.

In the parietes of xviii on *a* and *b* lines there are gaps in the musculature, the gap over the *a* line especially noticeable, no follicles recognizable in the gaps. Into the lateral gap the male deferent ducts pass, uniting just prior to entrance into the parietes. The *a* and *b* penisetal follicles, or muscle strands therefrom, reach into xxi and xxiii, and pass into the parietes separately, the lateral follicle on the median face of the prostatic duct. Setæ ca 2.5 mm long, maximum thickness slightly less than 10 μ , shaft in part undulating but not regularly. Tip widened, almost membranous, margins curved, rather spoon to scoop-shaped. Supposed prongs at margins of web may be an optical illusion. Ornamentation, continued for some distance down the shaft, of longitudinal rows of triangular teeth with a few smaller, more thorn-like projections, occasionally presence of teeth indicated only by marginal incisions. All ventral follicles of viii and ix are enlarged, muscular,

conspicuously protuberant into coelomic cavities. Setæ nearly straight or with a short ental portion slightly curved. Tip slightly clawed.

The spermathecal duct at first appears to be about as long as to slightly longer than the wider ampulla from which it is sharply marked off, an ental portion (less than half the length) which is widened, thick-walled, bulbous, receives on the lateral side the diverticulum. The slender ectal portion of the duct is sinuous or even very shortly looped in a regularly zigzagged fashion the loops covered over by connective tissue so as to make the duct look stouter and shorter than is actually the case. The diverticulum is shortly digitiform, with no externally recognizable demarcation into stalk and seminal chamber, an ental portion characterized by a slight spermatozoal iridescence or filled with gray or brown material.

Remarks—In this as well as other species of the genus, and also in *Pellogaster*, there may be visible on setal arcs, especially of preclitellar segments, transverse rows of small, dot-like markings, possibly sensory. These markings produce an appearance as of perichætine setæ and when setæ are retracted into the parietes, render estimation of intersetal relationships and determination of certain positions difficult.

The penial setæ of the present specimens are longer and definitely thicker than those measured by Stephenson, and ornamented. In absence of other and more significant differences identification as *trichochætus* is presumed to be correct.

Genus *Barogaster* Gates, 1939

Barogaster barodensis (Stephenson) 1914

Material examined —

Nowgong, Chhatarpur State, Bundelskand, Central India "Underneath stones by chota nullah 2 miles from Nowgong", August, 8 juvenile, 3 achatellate and 15 clitellate specimens. "Grain Market, Gola Bazar", August, 2 clitellate specimens E C Pahari

Chaubara, west of Nowgong, August, 4 clitellate specimens E C Pahari

External characteristics—Length (strongly contracted) 65–100 mm. Diameter 3·5 mm. Unpigmented, clitellum yellowish to brownish. Prostomium prolobous but a narrowly triangular area with base on groove marking prostomium off from 1 continued well towards 1/2, usually with a median furrow from posterior apex of triangle to 1/2. The triangular area may be deeply depressed, then appearing as a median groove.

Spermathecal pores on, in line with, or immediately behind 7/8 Female pore unpaired and median (16) Male pores minute, on juvenile and achi- tellate specimens slightly median to *b* lines, on clitellate specimens often slightly lateral to *b* lines Tips of one or two penial setæ are recognizable within each pore on juvenile specimens but not on others Male porophores are always low and flat, of variable shape but usually transversely placed, each including a single genital marking which is about on the *a* line Porophores may (or appear to) be united occasionally with genital marking areas of xviii or xvii

Genital markings are often circular and are always small Anterior to the clitellum markings are not on areas of noticeable epidermal thickening and hence may often be difficult of recognition Occasionally one or two markings are present on the presetal annulus of viii, on each side, just behind the spermathecal pores, but otherwise markings of viii are on the setal annulus, in *aa*, *ab*, or *bb* One specimen has two markings in *aa* on the setal annulus of ix A number have markings on vii (18)

Posteriorly markings are in a single transverse row on each segment, usually of one to three on xviii, as many as eight on the other segments On juveniles, rudiments of markings are recognizable only on xvii where they are in contact with 17/18, hence definitely postsetal On clitellate worms markings are always included within sharply demarcated, raised, transversely placed areas of considerable epidermal thickening in *aa* or *bb*, on xviii restricted to a middle portion of *aa* or rarely united with the male porophores (1 specimen with six markings) Intersegmental furrows may be unrecog- nizable or only doubtfully so in the region of the markings so that the areas may appear to transgress slightly onto anterior margins of succeeding segments Locations are as follows on xv (1), xvi (16), xvii (18), xviii (17), xix (8, of which one has this row only), one specimen with markings on xvii-xviii only, another with markings on xvi-xvii only

Internal anatomy —The typhlosole begins in xxiv (3), xxv (6) or xxvi (1) The "grid" is located as follows Ixviii-Ixxviii (worm of 146 segments). Ixx-Ixxxii (153) segments, lxx-lxxviii (165 segments), lxxi-lxxix (138 and 164 segments), lxxi-lxxxii (160 segments), lxxii-lxxx (147 segments), lxxii- lxxxii (164 segments), lxxiii-lxxxii (139 segments), lxxiii-lxxxiii (157 segments)

Lateroparietal trunks in five specimens pass up in xiii onto the dorsal face of the gut and unite just behind 12/13 to form the supra-oesophageal trunk which is recognizable anteriorly into ix In one worm the right trunk passes to the supra-oesophageal but the left turns under the gut and

becomes continuous with the extra-oesophageal. A short vessel may connect the two extra-oesophageals just in front of the calciferous glands of XII.

Follicles of ventral setæ of VII and VIII are not enlarged and setæ are sigmoid, apparently unornamented.

Penial setæ are almost straight or slightly curved, tips slightly narrowed and rounded. Ornamentation may be scarcely recognizable, apparently quite lacking rarely.

Remarks - The species has been known hitherto only from the types secured at Baroda.

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REVERSAL OF THE EFFECT OF ACETYLCHOLINE ON THE FROG HEART WITH NORMAL VAGUS ACTION; TETANISATION OF THE FROG HEART; GRADED RESPONSES IN THE FROG HEART; THE EFFECT OF ELECTROLYTE FREE-MEDIUM

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OBREŠHKOVÉ (1942) has found that the heart of a small crustacean *Daphnia magna* is stimulated by acetylcholine and physostigmine. The heart tissue of the dogfish, *Squalus acanthias* is relatively insensitive to the action of acetylcholine (Bozler, 1943). During experiments on the frog stomach in December 1944, and January 1945, animals were encountered, the stomachs of which did not respond to the action of acetylcholine. As this was an anomalous effect, it was decided to investigate the properties of other tissues from the same animals. The rectus abdominis was sensitive, but the heart showed certain anomalous effects. In the present paper a description of these effects is presented.

METHODS

The acetylcholine used was a B.D.H. product, and produced contraction of the dog stomach, guinea pig uterus, guinea pig gut, guinea pig stomach, rabbit gut, and dog intestine. In some frogs it produced its typical effects on the frog heart and stomach.

The beats were recorded *in situ*, and drops of acetylcholine solution were dropped on the heart; sometimes the heart was almost immersed in the acetylcholine solution, so that the whole animal was flooded. In this way the drug was absorbed from the tissues of thorax and abdomen, and reached the heart via blood stream also. In the second method the heart was perfused by a Syme's cannula.

RESULTS

Resistance to Acetylcholine

The stomach was completely insensitive to acetylcholine (1 in 10⁴ to 1 in 10⁶), even after treatment with eserine (1 in 10⁶ to 1 in 10⁸), although it responded to alternating current, direct current and potassium in the normal manner (over 12 experiments). This appears to be a natural atropine effect.

The hearts of these frogs also were refractory to the action of acetylcholine, in that, no inhibition was produced even after eserination. The stomach and the heart were resistant to the toxic action, of eserine in that they did not lose their irritability in high concentrations (1 in 10⁴); in this they resembled *Mytilus* muscle. The excitability of the stomach to alternating current was actually increased by high concentrations of eserine, though in others it was depressed. Stimulation of the vagus nerve in these animals produced complete standstill of the heart, but acetylcholine produced augmentation of the beats, especially with high concentrations (Fig 1 A, B).

Properties of Acetylcholine Inhibition

Effect of initial length.—Starling has shown that the energy of contraction is a function of the length of muscle fibres. Singh (1942) has shown that not only is there an optimum length for excitation, but also for inhibition. In the frog heart it was found that the inhibitory effect of acetylcholine increased with the length of the muscle fibres.

It was found that the inhibitory effect of acetylcholine depended upon the perfusion pressure (Fig 2). Thus in six experiments it was found that acetylcholine produced complete standstill of the heart at a perfusion pressure of 3 cm. of water, but none at $\frac{1}{2}$ cm. With intermediate pressures the effect was intermediate.

These results can also be demonstrated by gradual lowering of perfusion pressure; with the fall of pressure, the heart recovers from the effect of acetylcholine (Fig 1 E), thus simulating a phenomenon corresponding to the "vagus escape". The above experiments were controlled with saline only and by administration of atropine, as at high pressure, inability of the heart to overcome the pressure, simulates inhibition.

Adaptation.—In some experiments the heart recovered from the effect of acetylcholine, the experiment being performed at constant volume (Fig. 3 D, N). After the recovery, the beats were augmented before return to the normal. The recovery was due to adaptation and not destruction of acetylcholine as shown by the fact, that the heart had become refractory to

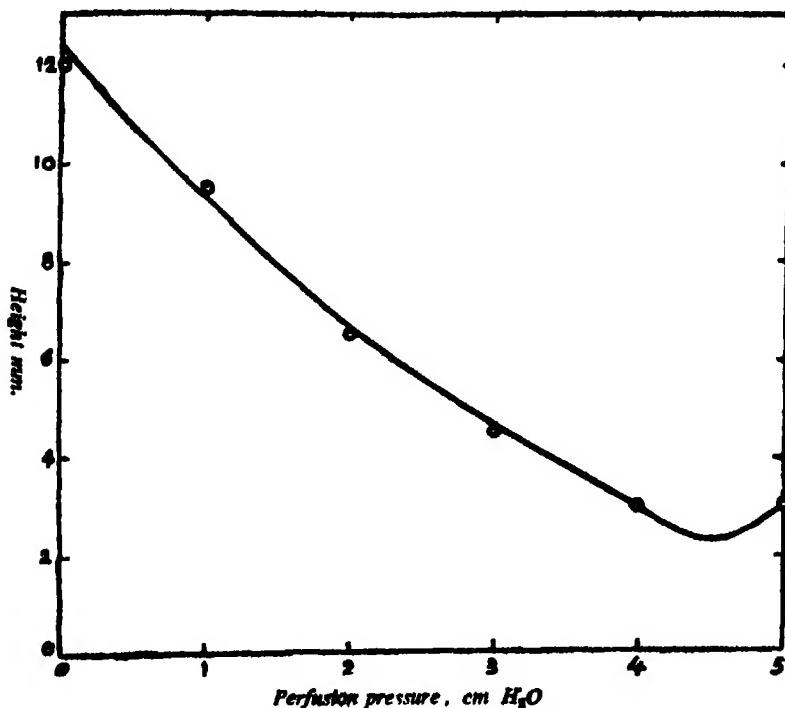


FIG. 2 Frog heart

Effect of perfusion pressure on the inhibition by acetylcholine, 1 in 10⁷. At high pressures the increase in excitability may counteract inhibition

stronger doses of acetylcholine. If the acetylcholine Ringer solution was alternated with acetylcholine-free Ringer solution, then the inhibitory effect was reproduced. These results resemble those on unstriated muscle (Singh, 1942).

Sometimes, acetylcholine produced partial inhibition but with each subsequent treatment after restoration to normal Ringer solution, the heart became less sensitive, so that ultimately acetylcholine failed to produce any effect. These results are similar to those found in *Mytilus* muscles, which were comparatively insensitive to potassium; when they were stimulated with potassium, adrenaline or acetylcholine, with each successive stimulation, the muscle became less sensitive, till finally it became insensitive.

Effect of potassium.—The classical effects described by Ringer were not observed, there was no prolongation of relaxation in the absence of potassium. There may be a temporary contracture in the absence of potassium (Fig. 3 M); but this disappeared with resulting augmentation of the beats.

In the absence of potassium, the magnitude of the beats was increased, and so also the inhibitory effect of acetylcholine (Fig. 3 G). In unstriated muscle, potassium in small concentrations antagonises the inhibitory effect of adrenaline, though by itself it produces inhibition (Singh, 1945) Excess of potassium caused inhibition also and increased the inhibitory effect of acetylcholine. The heart adapts to the inhibitory effect of potassium. Calcium and potassium together (double the normal concentration) diminished the inhibitory effect of acetylcholine, and sometimes changed it to an excitatory one

Effect of osmotic pressure—Increase in the osmotic pressure of the saline to 1 2-1 4 times normal diminished the inhibitory effect of acetylcholine and caused contraction In unstriated muscle, increase in osmotic pressure to 1 2-1 4 times normal diminishes the inhibitory effect of adrenaline (Singh, 1945)

Effect of sodium chloride deficiency.—Replacement of 20% of the sodium chloride of the saline by sucrose diminished the inhibitory effect of acetylcholine Isotonic sucrose solution caused contracture of heart muscle.

Stimulation by Acetylcholine and Potassium

In some hearts acetylcholine produced a contracture as in smooth muscle (Figs 3 F, L), the spontaneous contractions being abolished or diminished Replacement of the 20% or more of the sodium chloride of the saline with potassium produced contracture, which was abolished by electrical stimulation. Heart muscle, like unstriated muscle, thus, has two excitabilities Burridge stimulated the heart with 5 per cent potassium chloride

Tetanus in the Heart

It is generally believed that the heart muscle cannot be tetanised In the present experiments the heart could be easily tetanised (Fig. 3 B, 1 D) All stages of tetanus from intermittent contractions to complete tetanus could be produced (Burridge, 1920)

Effect of acetylcholine.—Acetylcholine (1 in 10^6 to 1 in 10^8) favoured tetanus, incomplete tetanus being converted into a complete one (Fig. 3, B C)

Effect of calcium.—Calcium opposed the tetanus (Fig 1 F) These results resemble those on the unstriated muscle (Singh, 1938) wherein it was found that in the presence of calcium, complete tetanus of the *Mytilus* muscle was converted into an incomplete one. With continued stimulation, the complete tetanus may become incomplete as may happen in the plain muscle (Fig. 3 A)

This change is hastened by calcium excess, suggesting that adaptation in the heart is due to liberation of calcium

In the absence of calcium the excitability of the heart is greatly diminished, but the beneficial effect of contraction is well marked. If calcium is in excess, then the beneficial effect of contraction is absent (Fig. 3 O). These results resemble those in smooth muscle (Singh, 1944a), and suggest that the staircase effect is due to the liberation of calcium.

The All or None Law

If the heart was naturally at standstill, or was brought to standstill by acetylcholine, then the responses produced by electrical stimulation were graded (Fig. 1, G, H, J). The contraction produced by the break induction shock was bigger than that produced by make (Fig. 1 G).

Electrolyte-free Medium

The heart continued to beat in an isotonic solution of sucrose for about 30 minutes, subsequently heart-block set in, so that the auricles beat regularly, and ventricles irregularly for about two hours. The electrolyte-free medium described by Singh (1944 x) was sometimes more and sometimes less efficacious than the isotonic solution, but once the heart was at a standstill in the isotonic solution, it was temporarily revived by the half tonic solution. The events resembled as in the plain muscle. There was at first depression of excitability, which was followed by a recovery (Fig. 1 K). The rhythm was slow and the relaxation prolonged. After cessation of spontaneous beats, the heart responded to electrical stimulation for about two hours.

DISCUSSION

The properties of the heart muscle, in many respects, resemble those of plain muscle. The action of the vagus and acetylcholine may be antagonistic. It was found that acetylcholine potentiated the response to electrical stimulation in the frog stomach (Singh, 1939), as well as in the heart. It is therefore possible that chemical transmission potentiates electrical transmission.

The above results differ from the classical results. It is probable that the membranes of these frogs had altered in composition, probably by a change in their calcium contents, and so these anomalous results are produced.

From the effects of electrolyte-free medium, it appears that as with the plain muscle, spontaneous contractions and those produced by electrical

stimulation must be due to ions within the muscle fibres. Calcium and potassium are necessary constituents of the external medium so as to neutralise the effects of sodium chloride.

SUMMARY

Frogs were encountered, the stomachs and hearts of which were refractory to the action of acetylcholine, even after eserine. In the hearts, the beats were augmented by acetylcholine, but diminished by vagus stimulation. They could be tetanised, and the responses with electrical stimulation were graded. The auricles and ventricles beat rhythmically but independently in the absence of electrolytes.

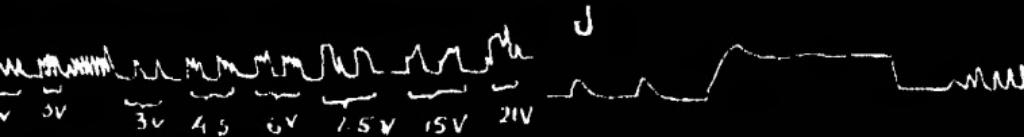
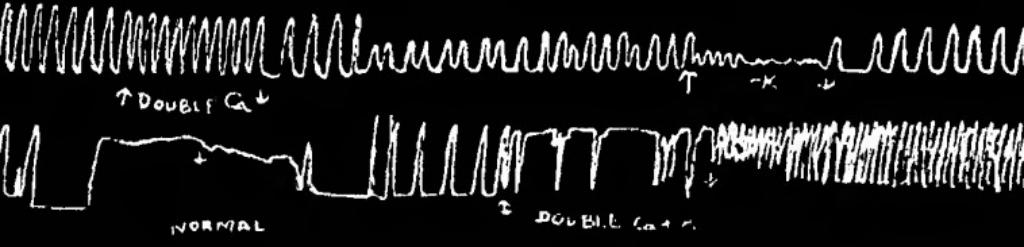
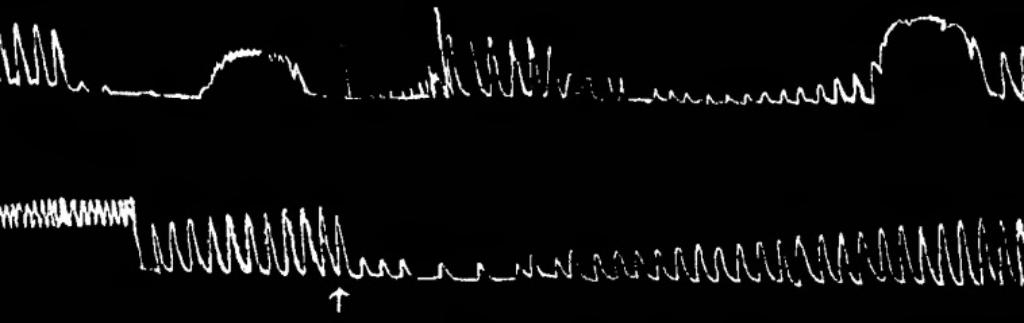
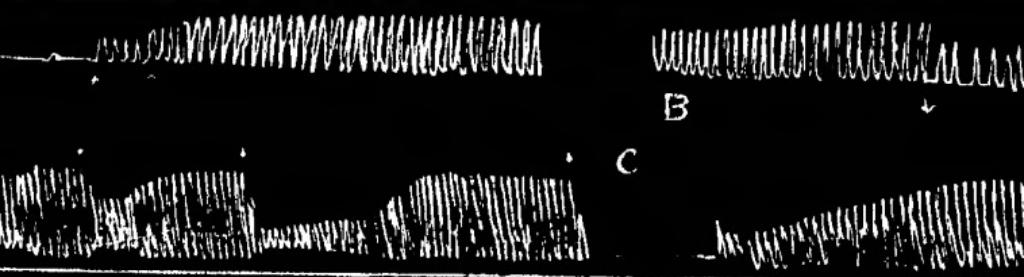
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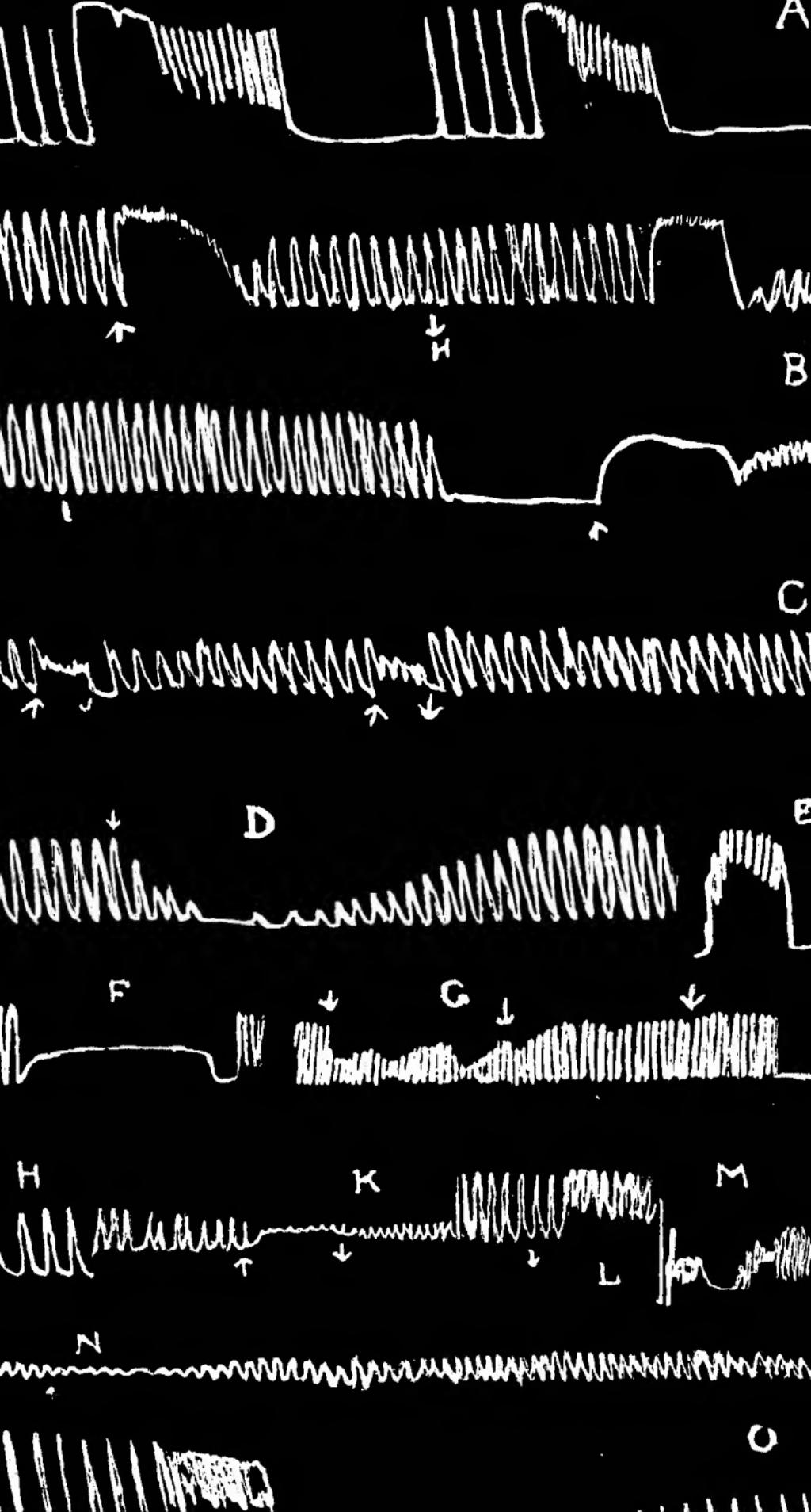
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EXPLANATION OF FIGURES

FIG 1 Frog heart—

- Stimulating effect of acetylcholine. The heart was at a standstill with an occasional small beat. Addition of $1 \text{ in } 10^7$ acetylcholine at the first arrow revived the heart. Further addition of $1 \text{ in } 10^6$ acetylcholine at the 2nd arrow augmented the beats still more.
- Stimulating effect of acetylcholine $1 \text{ in } 10^6$. Added at the first arrow and withdrawn at the 2nd.
- Dependence of the inhibitory effect of acetylcholine on the initial length of the muscle fibres. At the first arrow perfusion pressure raised to 5 c.c. water. Note the mechanical effect simulating depression, as the pressure was reduced the normal magnitude of the beats was recovered. At the 2nd arrow was added $1 \text{ in } 10^7$ acetylcholine, at the 3rd arrow $1 \text{ in } 10^6$, and at the 4th, $1 \text{ in } 10^5$. The inhibitory effect of acetylcholine disappears as the perfusion pressure is lowered.
- Tetanus produced by sending repeated induction shocks. Treated with $1 \text{ in } 10^6$ acetylcholine.
- The first contracture shows after-effects of tetanus. After a tetanus, the inhibitory effect of acetylcholine is antagonised, and the heart is hyper-irritable before return to normal. Acetylcholine $1 \text{ in } 10^7$, added at arrow; note recovery with lowering of perfusion pressure.





- F. Tetanus. First tetanus in Ringer 2nd tetanus with double calcium and potassium, 3rd tetanus (upper tracing) with double calcium only, note only intermittent contractions. 4th tetanus in the absence of potassium In the absence of potassium, the normal beats are augmented The ordinary Ringer was not a suitable solution for these hearts
- G. Graded responses First contraction by make induction shock, second contraction by break induction shocks 3rd contraction tetanus Afterwards make and break shocks The break shock contractions are bigger than make ones
- H. Graded responses Stimulation with direct current for 5 sec /10 sec
- J. Graded responses First two contractions by 1 3 v D C 3rd contraction by 14 v D C Note slow relaxation, as happens in the plain muscle with high voltages The heart is not injured as shown by resumption of intermittent contractions
- K. In isotonic sucrose. Note 1st effect (after the arrow) is a contracture, as happens in the plain muscle Then depression of excitability, as in the plain muscle Then recovery with slow relaxation The heart continued to beat for 35 min

FIG 3 Frog heart—

- A. Tetanus Heart not beating Single contractions by single induction shocks As the muscle adapts during tetanus intermittent contractions recover, as in the plain muscle
- B. Tetanus Lower figure, after acetylcholine, 1 in 10^6
- C. Incomplete tetanus
- D. Adaptation to acetylcholine, 1 in 10^7 added at arrow
- E. Contracture of the frog heart (at standstill by acetylcholine) by Ringer with double potassium and calcium.
- F. Contracture by 1 in 10^6 acetylcholine
- G. Effect of potassium At 1st arrow, 1 in 10^7 acetylcholine is added, slight variation in the inhibition due to variation in perfusion pressure 2nd arrow, restoration to normal 3rd arrow, potassium free Ringer solution, complete standstill produced by acetylcholine in the absence of potassium
- H. Note an extrasystole
- K. Contracture produced by potassium (20% of NaCl replaced with KCl)
- L. Contracture and augmentation produced by 1 in 10^6 acetylcholine
- M. Inhibition by 1 in 10^7 acetylcholine Thereafter potassium free Ringer Preliminary contracture which disappears with augmentation of the beats
- N. Adaptation to acetylcholine First arrow 1 in 10^7 acetylcholine, 2nd arrow 1 in 10^6 , 3rd arrow 1 in 10^6 and subsequently 1 in 10^4
- O. Same heart as in A Calcium content 4 times normal Incomplete tetanus only. The heart was not beating but contractions produced by single induction shocks After tetanus calcium-free Ringer Pronounced beneficial effect of contraction with repeated stimulation.

A COMPARATIVE STUDY OF AUTOTETROPOLOID AND DIPLOID TYPES IN MUNG (*PHASEOLUS RADIATUS LINN.*)*

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Received December 18 1944

THE successful production of an autotetraploid form in *mung* (*Phaseolus radiatus Linn*) by colchicine induced method has been previously described (Kumar and Abraham†) A few tetraploid plants were first obtained in 1941 Since then their successive progenies have been grown each season except for one namely 1943 when the entire crop was wiped out due to an extremely severe attack by insects. Fortunately, however, the seed kept in reserve helped to tide over the adverse period and continue the studies of further generations The autotetraploid type has reached the fourth generation The present study was undertaken to compare the performance of the autotetraploid with its diploid progenitor in respect of several important characters which are tabulated below

TABLE I Data from crop grown in 1942

Plant character	Tetraploid		Diploid		% increase (+) or decrease (-) over diploid	
	No. of plants observed	Average value	No. of plants observed	Average value		
1 Height in mm	..	85	391.1	92	485.4	- 19.4
2 Leaf						
(a) Length in mm	.	85	90.2	91	101.3	- 11.0
(b) Breadth in mm	.	..	98.0	..	99.7	- 6.7
3 Number of branches per plant	.	..	3.6	..	4.0	- 35.0
4 Number of pods per plant	.	90	41.5	..	129.9	- 68.0
5 Flowers						
(a) Petal (standard) length in mm	73	14.9	..	13.5	+ 10.4	
(b) Petal (standard) width in mm	..	19.3	..	17.0	+ 13.6	
6 Seed						
Yield per plant in grams	.	80	4.7	91	23.83	- 79.4
Germination percentage						

* The paper was read before the Joint Meeting of the Indian Academy of Sciences, Bangalore, and the National Academy of Sciences, United Provinces, held at Poona in December 1944

† 1 "Induction of Polyploidy in Crop Plants," L S S Kumar and A Abraham, Current Science, 1942, 2, No 3, pp. 112-14

2. "Study of Colchicine Induced Polyploidy in *Phaseolus radiatus L.*," L S S Kumar and A Abraham, Jour University of Bombay, 1942, 11, Pt 3, 30-36

Comparative Study of Autotetraploid & Diploid Types in Mung 267

From the above table it will be observed that except for flower size which has increased, all other characters namely height, size of leaf, number of branches, number of pods set and yield have been adversely affected in the tetraploid type. The percentage of decrease or increase over the diploid varies for different characters. Of the six characters studied, five have been adversely affected and only one shows favourable or *gigas* effect. The effect has been drastic particularly in respect of characters affecting the yield of plant.

TABLE II. *Data from crop grown in 1944*

Plant character	Tetraploid		Diploid		% increase (+) or decrease (-) over diploid
	No. of plants observed	Average value	No. of plants observed	Average value	
1. Pod					
(a) No. of pods per plant	18	7.0	17	9.5	- 26.2
(b) No. of seed per pod	"	4.0	"	9.7	- 59.8
(c) Breadth in mm	"	5.7	"	4.7	+ 21.3
(d) Length in mm	"	42.7	"	63.0	- 35.8
2. No. of flowers per plant	20	92.5	"	235.1	- 60.7
3. Weight of 100 seeds in grams	"	2.52	"	2.34	+ 7.7

The data recorded above which belongs to a different year shows that except for the increase in size of the seed and breadth of pod of the tetraploid the other characters exhibit a retrograde influence resulting from doubling of chromosomes. In respect of pod size, it is interesting to note that while the length of the pod has decreased, its breadth has increased. If this is compared with the effect of chromosome doubling on flower size (Table I), it is seen that both length and breadth have increased. Why there should be this differential influence in respect of the various characters and of the same character in respect of two separate organs need explanation.

The result of induction of tetraploidy in *mung* has not given the desired results of enhancing economic characters. Three years' observations go to show that the tetraploid is later in flowering, more susceptible to disease and extremely poor yielding. The reasons for low yield are, reduction in the number of branches and flowers produced, number of pods set, and number of seeds per pod.

If these results are considered with the result obtained in *rahar* (*Cajanus indicus* Spreng*) in which the induction of autotetraploid has resulted in

* A Preliminary Note on Autotetraploid *Cajanus indicus*, by L. S. S. Kumar, A. Abraham and V. K. Srinivasan, Proc. Ind. Acad. Sci. (In Press)

adversely affecting economic characters, it would appear that colchicine induced autotetraploidy is not a suitable method for improving the crops belonging to the pulse group. Probably colchicine induced allotetraploids may prove of greater use than autotetraploids of the type described above.

SUMMARY

Colchicine induced autotetraploid progenies belonging to two different years were compared with their diploid progenitor for various characters. Most of the characters of the autotetraploid exhibit a decrease over that of the diploid except in the case of petal size and pod breadth which have increased slightly. The unfavourable effect is rather drastic particularly in respect of characters affecting the yield of the plant.

Since very similar results have been obtained in *rahar* (*Cajanus indicus* Spreng), it is suggested that colchicine induced autotetraploidy may not prove to be a suitable method for improving the various pulse crops.

AN EMBRYOLOGICAL STUDY OF *ISOTOMA LONGIFLORA* PRESL

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Received May 1, 1945

(Communicated by Dr L N Rao, M.Sc., Ph.D., F.R.M.S., F.A.S.)

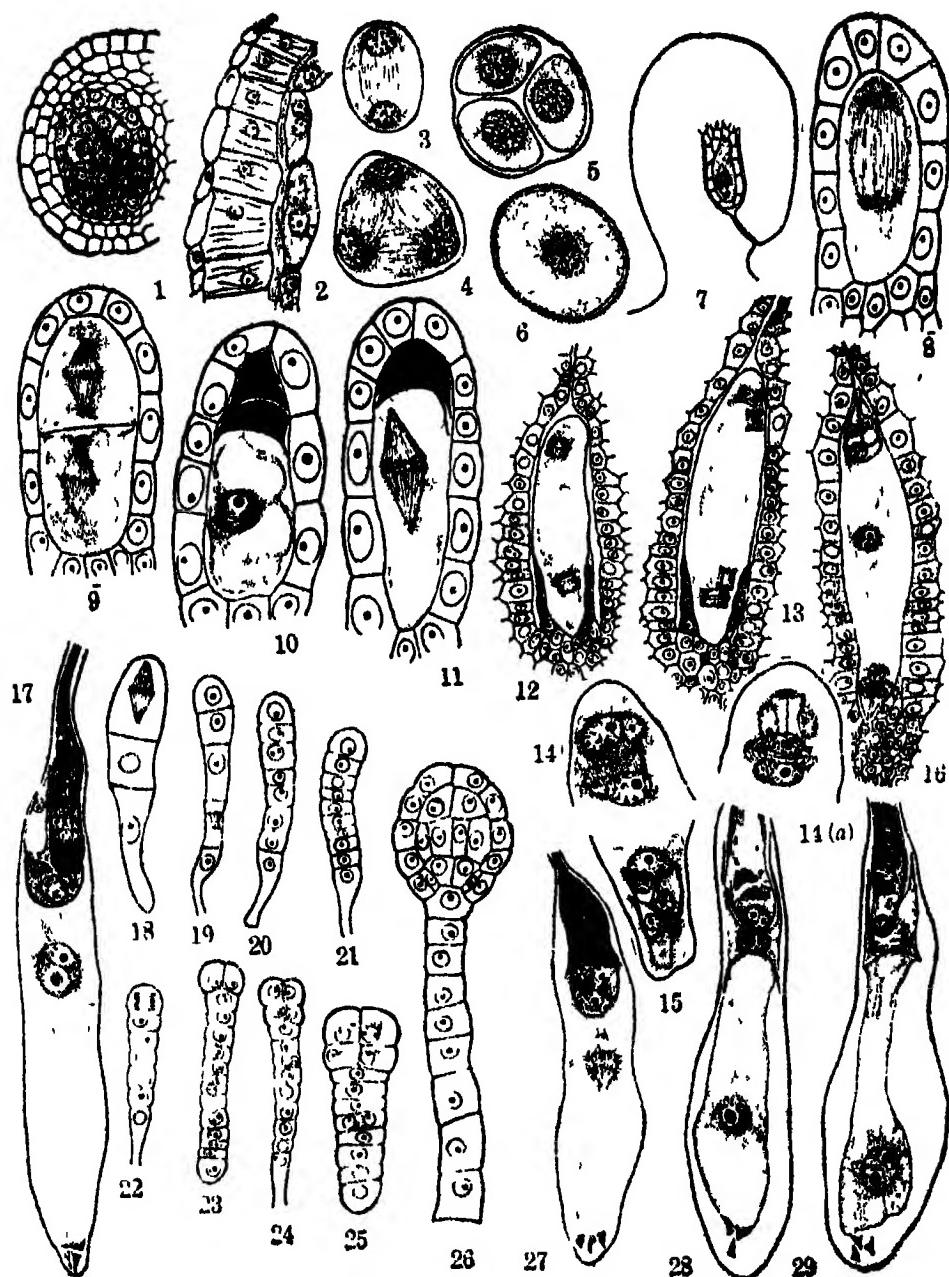
INTRODUCTION

The earlier literature relating to the embryology of the Campanulaceæ and the related family Lobeliaceæ has been reviewed by Schnarf (1931). Of the recent works mention may be made here of the paper by Rosen (1932) on the embryology of the Campanulaceæ and the Lobeliaceæ, and also of the papers on the Lobeliaceæ by Kausik (1935, 1938), Crete (1938), Hewitt (1939), Cooper (1942) and Maheshwari (1944 a, b). In this connection, we may note that Cooper's (1942) observations on *Lobelia cardinalis* L. that the synergids and the antipodals take part in the formation of the micropylar and chalazal haustoria respectively have been commented upon as erroneous by Maheshwari (1944 a, b).

The material selected for the present investigation, *Isotoma longiflora* Presl, is a West Indies species belonging to the tribe Lobelioidæ of the family Campanulaceæ of Engler (1897). This is an erect herb, found in wet situations and growing to a height of two feet, branching sparsely from the base, and possessing plenty of latex in all its parts. Leaves are linear dentate, flowers axillary solitary, or in groups of three, slightly zygomorphic; the corolla is tubular and narrow, and attains a length of about six inches with the limbs spreading horizontally, stamens are five in number with syngenesious anthers having hairs at the tips of connectives, ovary inferior, bicarpellary, syncarpous with indefinite anatropous ovules on a massive central placenta, the fruit is a capsule showing dehiscence by pores at the top.

MATERIAL AND METHODS

The material was collected in the Government Botanical Gardens, Bangalore, and fixed in Allen's modified Bouin. At the 70% stage in dehydration, the old ovaries were placed in Carnoy's fluid for one hour to facilitate slight hardening of the material and thus preventing the detachment of the ovules from the placenta. Subsequent treatment was according to the customary methods. Sections were cut ranging in thickness from



FIGS. 1-29

Figs 1 to 29 —Fig 1 Portion of a transverse section of an young anther showing wall layers, tapetum and the microspore mother cells $\times 560$ Fig 2 Portion of the anther wall at a much later stage showing the fibrillar endothecium, disorganised middle layer and the binucleate tapetum $\times 900$ Figs 3-5 Stages showing the first and second division in microspore tetrad formation, Fig 3 $\times 1260$, Figs 4 and 5 $\times 1800$ Fig 6 A mature trinucleate pollen grain $\times 1260$ Fig 7 Young anatropous ovule with massive integument and the first division of the megasporangium $\times 540$ Fig 8 Telophase stage in the megasporangium $\times 1800$ Fig 9 Second division in the formation of the linear tetrad $\times 1800$ Fig 10 Enlarging chalazal megasporangium and the degenerating upper three megasporangia forming the apical caps $\times 1800$ Fig 11 First division in the chalazal megasporangium $\times 1800$ Figs 12 and 13 Second and third nuclear divisions in the formation of the embryo sac, note the degenerating nucellar epidermis and the formation of the integumentary tapetum $\times 900$ Figs 14, 15 Micropylar and antipodal ends of the same embryo sac enlarged to show the late telophase spindles and the organisation of the antipodal cells $\times 1800$ Fig 14a Micropylar end of another embryo sac showing the relationship of the daughter nuclei in the organisation of the egg-apparatus $\times 1800$ Fig 16 Fully organised embryo sac showing the egg-apparatus, antipodal cells and the fusion nucleus $\times 900$ Fig 17 A late stage in fertilization showing remnants of the pollen tube, the zygote nucleus and the primary endosperm nucleus $\times 900$ Figs 18-22 Development of the filamentous proembryo, Fig 18 $\times 400$, Figs 19-21 $\times 560$, Fig 22 $\times 540$ Figs 23-25 Stages in the formation of the octant condition of the embryo, Figs 23, 24 $\times 560$, Fig 25 $\times 800$ Fig 26 Later embryo showing the differentiation into the dermatogen, periblaster and plerome $\times 900$ Fig 27 First division of the primary endosperm nucleus $\times 800$ Fig 28 Two-chambered embryo sac $\times 800$ Fig 29 Division in the upper chamber $\times 800$ (Original magnifications are indicated here, but the figures have been reduced to half in reproduction)

10 to 24μ Staining was done in Heidenhain's non-alum haematoxylin with eosine as counterstain for contrast

MICROSPORANGIUM

The wall of the young anther (Fig 1) shows three layers outside the tapetum. These are the outermost epidermis, next the endothecium which acquires fibrillar thickenings later (Fig 2), and lastly the middle layer which remains single and disorganises at the mature stage of the anther. The tapetal cells are uninucleate to start with, but later become binucleate (Fig. 2). The mother cells undergo the usual reduction divisions and form the tetrads of microspores (Figs 3-5). The separation of the microspores is effected by cleavage furrows which are initiated at the periphery (Fig 4). The tetrads are typically tetrahedral (Fig 5). The mature pollen grains are trinucleate at the time of shedding (Fig 6) and possess a rigid wall with spinescent projections on the surface. The intine appears as a thin and delicate membrane. Three germ pores are seen in the wall of the pollen grain.

DEVELOPMENT OF THE EMBRYO SAC

The ovule is anatropous and possesses a single massive integument (Fig 7) enclosing a small nucellus. The megasporangium undergoes the usual reduction divisions and gives rise to the linear tetrad (Figs. 8-10).

The upper three megasporangia soon degenerate, while the chalazal megasporangium enlarges to form the embryo sac (Fig. 10). The three nuclear divisions that follow in the functioning megasporangium are quite typical, and the eight-nucleate embryo sac is thus formed according to the normal type (Figs. 11-13 and 16). When the second of these nuclear divisions is in progress the epidermal layer of the nucellus is seen to be almost destroyed, except for a few cells at the base, and consequently, during further development, the inner epidermis of the integument comes to lie in direct contact with the embryo sac and becomes differentiated as the integumentary tapetum (Figs. 12, 13 and 16). The final organisation of the embryo sac also proceeds normally with the formation of the egg-apparatus, the three antipodal cells and the two free polar nuclei which fuse almost immediately to form the fusion nucleus (Fig. 16). It may, however, be noted here that the antipodal cells seem to proceed to organise themselves as cells slightly earlier than the egg-apparatus. Figs. 14 and 15 show the micropylar and antipodal ends respectively of one and the same embryo sac where it is found that the nuclei are still in the late telophase stage at the micropylar end, while the cell formation at the antipodal end is almost completed. Fig. 14a represents the micropylar end of another embryo sac where the late telophase spindles of the third nuclear division are seen, with the line of phragmoplasts along the equator quite evident and thus indicating clearly the separation of the daughter nuclei showing their future relationship in the egg-apparatus, namely that the synergids are formed from two sister nuclei while the egg and the upper polar nuclei are similarly formed from the other set of sister nuclei. This is in conformity with the view expressed by Porsch (*cf.* Maheshwari, 1937) with regard to the homology of the angiosperm embryo sac.

In the mature embryo sac, at the time of fertilization, the antipodal cells are seen as degenerating cells (Fig. 17). The pollen tube enters the embryo sac by destroying one of the synergids. Both syngamy and triple fusion occur as normal processes during fertilization (Fig. 17).

EMBRYO

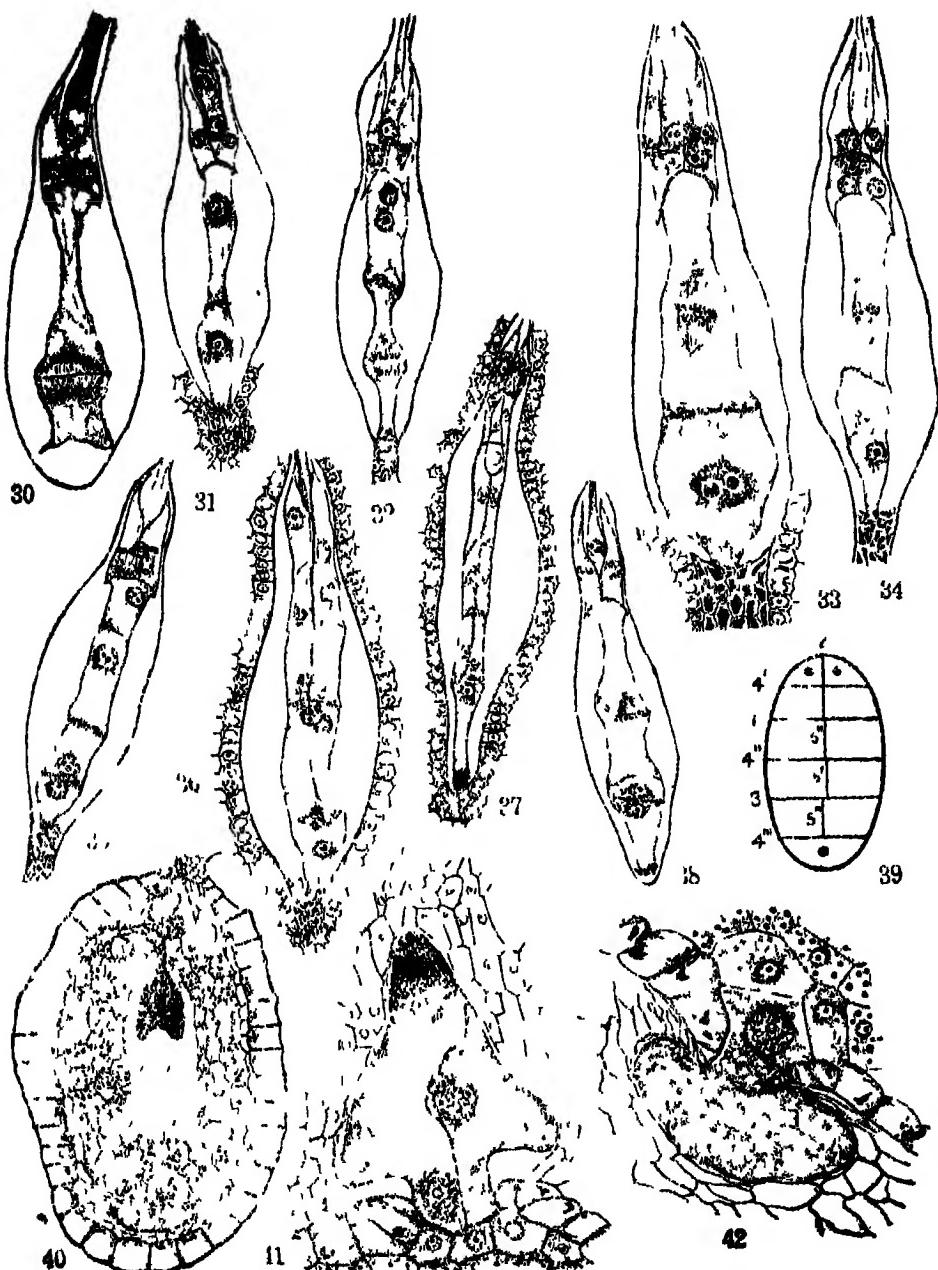
The zygote elongates rapidly and becomes tubular (Fig. 36). The first division in the zygote takes place only after the endosperm has passed through its initial development (Fig. 37). After a series of transverse divisions a filamentous proembryo of 10 to 12 cells (Figs. 18-21) is formed. The terminal cell of this proembryo is the embryonal cell which, after the first vertical, and the second and third transverse and vertical walls, forms the octant stage (Figs. 22 to 25). With further growth, the embryo becomes large and spherical in which the dermatogen, periblere and plerome become

next differentiated (Fig. 26). The first cell of the suspensor becomes wedged into the base of this spherical embryo and gives rise to the hypophysis. The development of the embryo thus conforms to the *Capsella*-type. The late embryo is typically dicotyledonous with the stem tip arising in the deep notch between the two cotyledons (Fig. 40).

ENDOSPERM

The primary endosperm nucleus undergoes the first division in the upper one-third of the embryo sac (Fig. 27). Following this a transverse wall is laid here to form a small upper primary micropylar chamber and a much larger lower primary chalazal chamber (Fig. 28). The upper chamber then divides by a vertical wall to form two cells placed side by side (Figs. 29 to 31) while the nucleus of the lower chamber descends almost to the base of the embryo sac where it divides followed by the formation of a transverse wall (Figs. 28 to 30). Thus a small lower cell and a larger middle cell are formed as shown in Fig. 31, and the embryo sac, therefore, has at this stage three distinct regions. Subsequent divisions take place in all these three regions of the embryo sac, the nuclear divisions being either almost simultaneous in all the regions, or proceeding ahead in certain portions (Figs. 32 to 34). With the completion of these divisions the embryo sac shows six major tiers, the uppermost two consisting each of two cells arranged side by side, and the remaining four tiers having only one cell each (Figs. 34 and 35). The lowest cell in this series does not divide any further, but takes part directly in the formation of the chalazal haustorium which later becomes a prominent bulbous structure with a single large nucleus (Figs. 36, 37, 40 and 42). Similarly, the two cells forming the uppermost tier in the embryo sac also become conspicuous and form the micropylar haustorium, the two cells becoming long and tapering distally, and also later developing a prominent lateral bulge or hump (Figs. 36, 37, 40 and 41). The remaining four central tiers of the embryo sac give rise to the endosperm tissue (Figs. 36, 37 and 40). Here, it is to be noted that it is only the first tier which is two-celled, while the other three are made up of only one cell each (Figs. 34 and 35), but in each of the latter also a vertical wall is next laid so that the primary endosperm cells soon become arranged in two longitudinal rows (Figs. 36 and 37). With further development the endosperm tissue increases rapidly in its bulk and fills the whole cavity of the seed inside the seed-coat (Fig. 40).

A slight departure from the course of development detailed above is met with not infrequently in certain ovules found either in the same ovary or in different ovaries. This is shown in Fig. 38 where it is seen that the nuclear spindle for the division in the large central cell formed after the second



FIGS 30 TO 42

Figs 30 to 42—Fig. 30 Division in the lower chamber $\times 800$ Figs 31-37 Stages in the development of the endosperm and the separation of the micropylar and chalazal haustoria Fig. 31 $\times 560$, Fig. 32 $\times 800$, Figs 33-35 $\times 560$, Fig. 36 $\times 400$, Fig. 37 $\times 560$ Fig. 38 A stage in the development of the endosperm according to the *Phyteuma*-type described by Rosen $\times 560$ Fig. 39 Diagrammatic scheme showing endosperm development Fig. 40 Longitudinal section of a mature seed showing the thickened walls of the epidermis, starch-filled endosperm, with the micropylar and chalazal haustoria and the dicotyledonous embryo $\times 800$ Fig. 41. The two-celled micropylar haustorium at a late stage in the seed $\times 900$ Fig. 42 The uninucleate single celled and bulbous chalazal haustorium, also at a late stage 1260 (Original magnifications are indicated here, but the figures have been reduced to half in reproduction.)

transverse wall at the base of the embryo sac is oriented almost transversely, with the result that a vertical wall is laid here, and not a transverse one as in the method of endosperm development described above. Finally, however, after subsequent divisions in the embryo sac, the primary endosperm cells become arranged in two longitudinal rows as in the first method.

In the mature seed (Fig. 40) the outer epidermis forms a rigid and hard protective covering, with the inner and radial walls of the cells extremely thickened. The endosperm tissue fills the entire seed cavity and its cells are rich in stored starch grains. The micropylar and chalazal haustoria are still very conspicuous and appear as darkly staining structures with prominent nuclei. The embryo with its slender suspensor almost quite shrivelled up lies deeply buried in the large mass of the endosperm tissue.

CONCLUSIONS

In the course of the present investigation it has been possible to get a complete and continuous series of stages illustrating endosperm development, and, as already stated, we find that there are two rather distinct and separate types in this development. Both these types are met with equally commonly, occurring as they do each in approximately 50% of the ovules, either in the same or different ovaries. According to the first of these types, which has been dealt with at some length in the paper, the scheme of development, although apparently resembling the *Phyteuma*-type of endosperm development described in the Campanulaceæ by Rosen (1932), appears to differ from this type in certain essential respects in the initial stages. On the other hand, it is to be noted here that the second type referred to briefly in this paper is found to agree closely with the *Phyteuma*-type of Rosen. In view of the fact, therefore, that a course of development of endosperm which seems to represent a marked departure from the *Phyteuma*-type also occurs in *Isotoma longiflora* Presl, we think that it should be referred to a separate, but rather closely related type. Further, it is also met with in quite a large number of observed cases, in fact in about 50% of the ovules. It appears,

therefore, quite reasonable to assume that this scheme of endosperm development is a distinct one which we may designate as the *Isotoma*-type.

Rošen (1932) has also described a second scheme of development in the Campanulaceæ, the *Codonopsis*-type. According to him, an important difference between this and the *Phyteuma*-type is that the chalazal haustorium is two-celled in the former, while it is made up of only a single uninucleate cell in the latter. He further states that the course of endosperm formation according to the *Codonopsis*-type is comparable to that in the *Scutellaria*-type, and that in the *Phyteuma*-type there is seen a combination of the *Scutellaria*- and the *Ericacea*-types of development. It is, therefore, interesting to consider here the course of endosperm formation in the closely related family Lobeliaceæ. According to Maheshwari (1944 b), in *Lobelia trigona* Roxb. the primary chalazal chamber of the embryo sac divides by a vertical wall after which both the cells thus formed undergo at least one more division by a transverse wall to give rise to the chalazal haustorium* and an upper set of two cells, the latter contributing also to the endosperm tissue along with the cells derived from the divisions of the upper primary chamber. This course would then correspond to the *Scutellaria*-type. Therefore, the relationship between the families Campanulaceæ and the Lobeliaceæ is thus also found to be very close as indicated by the courses of development seen in the members belonging to these two families. Further, as Maheshwari (1944 b) also suggests, according to the observations of Hewitt (1939), the type of development in *L. amoena* appears also to be similar to that in *L. trigona*. Hewitt finds, to quote Maheshwari (1944 b), that in *L. amoena* "two cells at each end of the eight-celled endosperm develop into large micropylar and chalazal haustoria, the remaining four cells developing into the large central mass of endosperm". In yet another species of *Lobelia*, *L. triplata* Buch-Ham, the same course of development seems to proceed according to some very recent observations (unpublished) that we have now been able to make.

With regard to the systematic position of *Isotoma*, we may note here that according to Engler (1897) it finds a place in the tribe Lobelioidæ of the family Campanulaceæ. According to Hutchinson (1926), on the other hand, the genus is placed in a separate family, Lobeliaceæ, along with the typical genera like *Lobelia*, *Pratia* and others under the order Campanales. Thus we see that Hutchinson has split the single family Campanulaceæ of

* It may be noted here that it is likely that this particular stage depicting the exact mode of separation of the chalazal haustorium in *Lobelia trigona* Roxb. was missed by one of us (Kausik, 1935). We now think that a transverse wall probably occurs as stated by Maheshwari (1942 b).

Engler into separate and distinct families, the Campanulaceæ and the Lobeliaceæ. Taking the gross external morphological features, the genus *Isotoma* seems to share many taxonomic characters in common with any typical member of the Lobeliaceæ, as for example *Lobelia*. But when we consider the greater details relating to the Embryology of *Isotoma longiflora* Presl, especially the course of endosperm development we think that there is a very close correspondence between this and the forms like *Phyteuma scheuchzeri*, *Campanula carpathica*, *C. patula*, *C. rotundifolia*, *Specularia speculum* and *Adenophora* sp studied by Rosén (1932). It may, therefore, be suggested here that *Isotoma* is probably to be regarded as a form linking the typical Lobeliaceous genera with the forms included under the Campanulaceæ, but finding a place, on embryological grounds, in the latter family, and the close relationship between these two families is thus also again very evident.

In conclusion, we wish to thank Mr S. N Chandrasekhara Iyer, Systematic Botanist, Coimbatore, for kindly determining the species. We are also thankful to Dr L N Rao, Professor of Botany, Central College, Bangalore, for the many courtesies extended to us.

SUMMARY

- 1 The anther shows a wall with only three layers, the tapetum becomes binucleate, pollen grains are tri-nucleate at shedding stage
- 2 The ovary has innumerable anatropous ovules attached on a massive central placenta. Megasporogenesis proceeds normally and the embryo sac is formed according to the normal type.
- 3 The embryo is formed according to the *Capsella*-type
- 4 Endosperm is *ab initio* cellular, and is formed according to the *Phyteuma*-type described by Rosén
- 5 A slight departure from the above type is met with in a large proportion of ovules, and since this is also quite a distinctive one, it may be regarded as a type, the *Isotoma*-type.
- 6 A reference to the systematic position of the genus *Isotoma* is made in the paper.

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A RARE MUTANT IN DROSOPHILA

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INTRODUCTION

In the course of a study on egg production in *Drosophila pseudo-obscura*, a Pointed snapt (P sp.)* female was observed to lay along with her apparently normal eggs, a certain number of peculiarly shaped eggs. These eggs when separately incubated yielded 12 females 3 of which on testing, proved to be abnormal layers. It was assumed therefore, that a mutation affecting egg structure had been recognised and since the only visible change from which its presence could be detected was through modified filaments—the two outgrowths on the eggs—it was decided to name this mutant as *Filament* (F.). *Filament* has since been isolated from the *Pointed snapt* stock in which it was first discovered and hence the question of *Filament* condition being due to one of the manifold effects of *Pointed* and *snapt* genes does not arise.

Filament forms the first *dominant* egg mutant to be described in *Drosophila* and possibly in all animals. The only other egg mutant known to science was discovered in 1937 in *Drosophila funebris* by Crew and Auerbach (1937). It was called as "spheroidal" (sp.) by them and behaves as a simple *recessive*. Unfortunately, due to extremely low fecundity and still lowered fertility of *spheroidal* females, a thorough investigation of this interesting mutant could not be carried out. Fortunately, such is not the case with *Filament*, whose fairly high fecundity and fertility has made possible this detailed study.

SPHEROIDAL vs. FILAMENT

(1) *Spheroidal* females have low fecundity and very much lowered fertility. Such is not the case with *Filament*. The egg production of *Filament* females in certain instances, closely approaches that of normal flies.

(2) *Spheroidal* eggs are shorter and broader. They are dwarfish in appearance. *Filament* eggs like *spheroidal* though shorter, are at the same time unlike *spheroidal*, narrower than normal eggs.

* A fly having narrow and pointed wings. If longitudinal vein short.

(3) Filaments of *spheroidal* are shorter and thinner. They may be very much reduced, but are *always* present. *Filament* filaments on the other hand, show not only a reduction in size, but also various degrees of fusion, as well as reduction coupled with fusion, of the filaments. Filaments may even be *totally absent*.

(4) *Spheroidal* females are known to lay particularly towards the end of their laying period, a certain percentage of pale, yellowish, transparent eggs, which may easily be overlooked in counting, as they do not strike the eye so prominently as normal eggs. These eggs seldom, if at all, hatch. Such pale, transparent eggs are also met with in eggs laid by *Filament* females; they are occasionally as frequent at the beginning of a laying period as at the end of it. 102 incubated eggs yielded 41 flies (20 ♂♂ and 21 ♀♀) showing their fair viability.

FILAMENT EGGS

(1) Size — *Spheroidal* eggs have been shown by Crew and Auerbach (1937) to be both broader and shorter than normal eggs. To see whether any such significant variation exists between *Filament* and normal eggs, measurements were made of the lengths and widths of 451 *Filament* and 150 *Wild type* eggs laid by 20 *Filament* and 10 *Wild type* flies of varying ages. The definitions of these dimensions are given in Fig. 17. Following are the results —

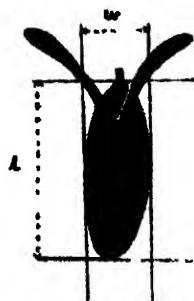


FIG. 17 Diagram showing the Definitions of Egg Size
 w = width l = length

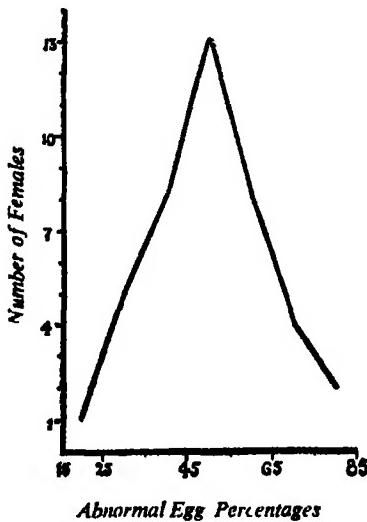
	Wild type	Filament	Difference
Length . .	32.55 ± 0.0952	32.14 ± 0.0802	$-0.41 \pm 0.1345\uparrow$
Width . .	12.15 ± 0.0334	10.80 ± 0.0140	$-1.35 \pm 0.0555\uparrow$

N.B.—Above figures are in units of Zeiss eye-piece micrometer 1 unit = 14 μ .

↑ Highly significant.

The differences are both statistically significant, showing that as compared with normal, *Filament* eggs are both narrower and shorter.

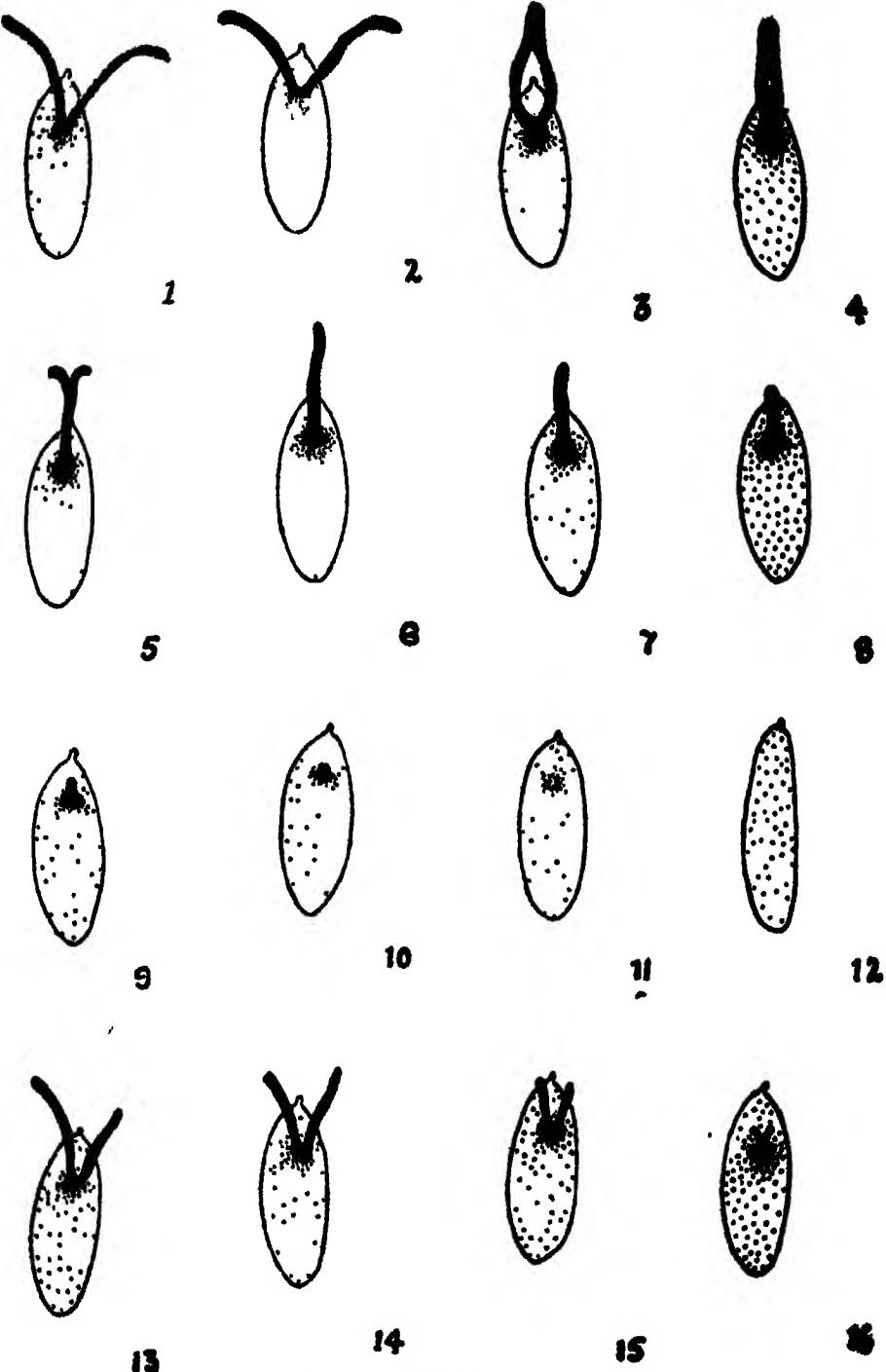
(2) *Shape* - The eggs laid by genetically *Filament* flies are not all alike in appearance. Only a small fraction of the eggs laid by the original *Filament* female were really abnormal looking. The others could not be distinguished externally from the normal for the species. The percentage of abnormal eggs vary from female to female. On an average, about 50% of the total eggs laid are abnormal. The frequency distribution of abnormal egg percentages obtained from flies after 8 generations of inbreeding is given in Graph 1.



GRAPH I. Graph showing the Frequency Distribution of Abnormal Egg Percentages found in 41 *Filament* Females

The difference in shape between the normal and the abnormal *Filament* eggs is very pronounced, the normal *Filament* eggs, as mentioned already, look exactly like the normal of the species. They are oval in shape and have two arm-like outgrowths arising separately and at about the same level towards the anterior poles of the eggs. The abnormal *Filament* eggs on the contrary, show a good deal of variation especially in the origin, shape, number and size of the filaments. An arbitrary classification based on the characteristics of the two filaments is given below.

(a) "*Normal*" (Fig. 1).—These resemble very closely the wild type eggs and cannot be distinguished from them.



FIGS. 1-16 Filament Egg Types

(b) "Fused"—Fig. 3 shows a typical "Fused" *Filament* egg. Eggs in this class are marked by a latero-median fusion of the two filaments. The fusion may be slight (Fig. 2)—confined to the bases of the filaments only, or nearly "complete" as in Fig. 5. Between the two extremes, various degrees of "Fused" eggs may be found. The free ends of partially fused filaments usually stick together (Fig. 4).

(c) "1-Filament"—When the fusion of the two filaments is "complete", i.e., involves the whole length of the two filaments "1-filament" condition is obtained (Fig. 6). The single filament so arising, occupies a median position and resembles very closely the filament normally found in the eggs of *Drosophila quinaria* and *Drosophila transversa* described by Sturtevant (1921). In thickness sometimes, it shows a greater resemblance to the median filament of *Drosophila quinaria* being as thick as, or slightly thicker than a normal one. In some cases, it is twice as thick as normal, when it approaches the condition obtained in *Drosophila transversa*. It may be equal to a normal filament in length (Fig. 6) or may show any degree of reduction in length (Figs. 7-10).

(d) "No-Filament"—These eggs unlike the other *Filament* eggs have a drawn out anterior end; as a result, the anterior region becomes narrower than the posterior (Fig. 12).

In addition to the above types, eggs with only reduction in size of one or both of the filaments (Figs. 14-16) have been occasionally met with. Eggs of this type are usually rare.

8,331 Eggs collected from 21 *Filament* females and graded according to the plan outlined above, give the following distribution:

Grade of Filament eggs	No of eggs	% of the total
(a) "Normal"	3,247	38.97
(b) "Fused"	976	11.72
(c) "1-filament"	2,297	27.57
(d) "No-filament"	1,811	21.74

(3) *Genetic Constitution*.—To find out whether the genetic constitution of the eggs is responsible for the observed difference in egg shape and size of eggs laid by *Filament* females, eggs collected from 20 (not virgin) *orange* (or) *Filament* females remated to *Wild type* (*Big Bear*) males, were divided into the various categories as "Normal", "1-filament", "Fused", etc., and separately incubated. The emerging flies will be of two types:

- (a) Flies derived from eggs fertilized by *orange* sperms
- (b) Flies derived from eggs fertilized by *Wild type* sperms

The former can be easily distinguished from the latter, by their *orange* eye colour. The latter would have the *Wild type* eye colour. Both *orange* and *Wild type* daughters were "tested" for *Filament*.

If *Filament* is recessive, only the *orange* females will show that character. If on the other hand, *Filament* is dominant, *Filament* will be found in both *orange* and *Wild type* daughters. Secondly, if the "Normal" *Filament* eggs are genetically *Filament*, then some of the flies emerging from "Normal" eggs are expected to be *Filament* (only some of the *orange* females, if *Filament* is recessive, or some from both *orange* and *Wild type* flies, if *Filament* is dominant). Table I gives the results obtained.

TABLE I
Genetic Constitution of *Filament Egg Grades*

Grade of <i>Filament</i> eggs from which the flies were obtained	Orange		<i>Wild type</i>	
	No. of females tested	No. F	No. of females tested	No. F
"Normal"	31	3	22	2
"Fused"	22	10	3	1
"1-fil"	8	6	16	3

Filament females were obtained in both *orange* and *Wild type* daughters showing that *Filament* is a dominant character. Some of the flies obtained from "Normal", "Fused" and "1-filament" eggs were *Filament*, which shows clearly that the shape of the eggs is not necessarily determined by the genetic constitution of the eggs. Even apparently normal looking eggs may be genetically *Filament*. This means that the variation in egg shape and size which we observe in *Filament* eggs is purely due to the poor "penetration" of the gene.

(4) *Hatchability*—The fertility of *Filament* females was found to be low, particularly when the output of abnormal eggs of the extreme types (Figs 9, 10 and 12) was high. The suspicion therefore arose that probably such eggs never hatched. A small-scale experiment was devised to look into this point.

About 15 well-fed *Filament* females (approximately of the same age) were mated to their brothers, who had been previously kept away from the females for 2-3 days to ensure ready mating (D.I.S. No 5). All the

eggs laid by these females in the first three or four days were collected on spoons, divided into the five different categories as "normal", "fused" "1-filament" and so on, and separately incubated. After 36-48 hours the spoons were re-examined and the unhatched eggs were counted. Results are given below in Table II

TABLE II
Hatchability of Filament Egg Grades

Grade	No of Eggs kept	No Hatched	% Hatchability
"Normal"	605	491	81 16
"Fused"	400	258	68 50
"1-filament"	745	330	44 30
"1-fil short"*	36	7	18 44
"No-filament"	459	0	0 00
Control—Normal	1153	1 133	98 27

* Extreme "1-filament" (Figs 9 and 10)

As all the females used were of approximately the same age, well-fed before mating, and only the eggs laid within the first 4 days were utilised for testing, the possible effect of aging of females on hatchability (which is considerable in these flies) might be ignored. The results show a definite and significant drop in hatchability as the egg shape shifts away from the normal. Thus "Normal" *Filament* eggs are highly viable as compared with "Fused" and "1-filament", whereas "No-filament" eggs which show the greatest departure from the normal mean shape and size, do not hatch. That this total failure of "No-filament" eggs to hatch is not due to any external factors such as total immersion of the eggs in the food which is possible, in the absence of the supporting filaments (which apparently prevent them from sinking into the food) was shown by the non-hatching of "No-filament" eggs even when collected on moistened blotting paper and transferred to smooth glazed non-yeasted food surfaces.

The results, therefore, indicate the existence of a definite relationship between egg-shape and hatchability. Such a relation has been observed in pullets where various authors (Dunn, 1922; Jull and Hayes, 1925; McClelland, 1931; Warren, 1934; Funk, 1934) have shown an inverse variation in hatchability percentages as the egg-size shifts away from the normal mean, but has not so far been reported in insects, where the eggs show, ordinarily, less departure from mean shape and size.

FECUNDITY AND OVARIAN RHYTHM

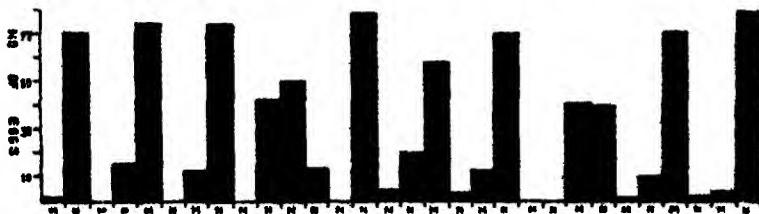
Following precautions were taken in obtaining the females used for the experiment

To start with, pair matings of *Wild type* (which served as Controls) and *Filament* flies were made. Flies were removed daily to fresh food vials to prevent overcrowding. 16 Pairs of *Wild type* and 16 pairs of *Filament* flies obtained from the above cultures, were then allowed to lay eggs on paper spoons; these spoons were changed daily at 9-30 A.M. Eggs collected on a particular day (third) were then divided into lots of 35 and distributed among vials ($3'' \times 1''$) containing approximately 1" of standing Schmidt and Offermann *Drosophila* food (D.I.S No 5) prepared the same day. The eggs were incubated at a constant temperature of $23.5 \pm 5^\circ C$. Of the hatching flies, 19 *Control* and 27 *Filament* females emerging at the same time (and therefore, approximately of the same age) were selected and *without* etherisation placed in $4'' \times 1''$ vials, with a male in each vial. Subsequently dead males were replaced by fresh ones.

Spoons were prepared at least 6 hours before actual use—the spoons for the morning transfer being prepared the previous night. All operations were carried out in the constant temperature room ($23.5 \pm 5^\circ C$). Great care was taken to avoid the least possible disturbance to the flies while changing the spoons. Spoons were changed twice a day (9-30 A.M. and 9-30 P.M.). Separate records of eggs laid by each female were maintained. A sample of the records so obtained is given in Graph II.



GRAPH II (i) A Sample Egg Production Record of a Filament Female



GRAPH II (ii) A Sample Egg Production Record of a Wild Type Female

RESULTS

A comparison of *Filament* and *normal* egg records clearly showed:

(1) The presence of the characteristic phenomenon called as "rhythm" which consists in periods of high laying alternating with periods of little or no laying [observed for the first time in *Drosophila pseudo-obscura* by Shapiro (1932) and later on confirmed by Dobzhansky (1935)] in both. This means that *Filament* does not radically change the egg-laying behaviour of the flies as to affect their rhythmic mode of egg production and laying. It is possible however, that the rate of egg production may undergo change without the rhythm being so affected. This point will be taken up later.

(2) *Filament* flies on the whole produced fewer number of eggs than *Controls*. While a *Control* female laid on an average a total of 906 eggs within a period of 15 days, the average number of eggs laid by a *Filament* fly within the same period, was only 767. Besides, the controls took on an average 22.4 *actual* laying periods (1 period = 12 hrs.) to deposit their 906 eggs as compared with 25.8 laying periods taken by *Filament* flies for depositing a comparatively lesser number (767) of eggs.

The evidence here suggests that the action of *Filament* is to *slow down* the normal function and activity of the ovaries. The ovaries thus arrested, would not only produce fewer number of eggs, but also would take a longer time to do so. To find out (1) whether *Filament* action starts right at the outset of egg production, or at a much later stage and (2) whether or not the effect, once started, persists unmodified, or with increasing or decreasing intensity throughout succeeding stages, the following analysis was undertaken.—

The egg records of individual flies were divided into periods of active and periods of little or no-layings. The total eggs laid between two successive periods of no-layings, gave the total eggs produced by the ovaries in that batch. In this way, batch-outputs of each fly, as batch followed batch, were computed; from which the mean outputs for various batches were calculated. The mean batch-outputs of *Filament* and *Control* flies so obtained, were then compared and tested for significance, using Fisher's method of comparing two means. It may be noted here, that we have taken batch-outputs, instead of period-outputs for purposes of comparison; because, the period-outputs give no indication of the state of ovarian activity as they are dependent upon (1) the total of the batches to which they belong and (2) the total eggs already laid in an immediately

preceding period or periods. The batch-outputs on the contrary are independent of these two factors and hence should give a truer picture of ovarian activity. The results are given below in Table III.

TABLE III

Comparison of Successive Mean Batch-Outputs of Filament and Control Flies

Batch No	Control		Filament		Difference (a-b)
	No of ♀ ♀	Mean batch output (a)	No of ♀ ♀	Mean batch output (b)	
1	33	79 12 ± 3.08	41	75 15 ± 2.98	3.97
2	33	87 09 ± 2.12	41	83 00 ± 3.37	4.09
3	33	91 18 ± 2.50	41	83 71 ± 2.53	7.47*
4	33	93 61 ± 2.63	41	78 20 ± 2.36	15.41†
5	32	88 06 ± 2.20	41	77 27 ± 2.58	10.79†
6	32	88 72 ± 2.74	40	76 18 ± 3.14	12.54†
7	32	84 25 ± 2.51	36	74 08 ± 3.04	10.17*
8	31	81 45 ± 3.31	33	72 33 ± 3.33	9.12
9	30	80 10 ± 3.13	29	66 17 ± 3.09	13.93†
10	26	77 12 ± 2.82	24	64 33 ± 2.52	12.79†
11	16	78 44 ± 3.46	13	62 77 ± 3.53	15.67†
12	8	72 63 ± 4.88	6	61 00 ± 4.18	11.63†

Figure mentioned after \pm is S E

* Significant at 5% level

† Highly significant

It can be seen from Table III that no significant differences exist in mean batch-outputs of *Filament* and *Control* flies in the first two and eighth batches, whereas, in all other batches, the differences are significant. This would mean that (1) *Filament* action does not start at the beginning of egg production. Physiological factors probably intervene at this stage which are able to successively overcome or check the influence of *Filament* gene. *Filament* action, therefore, is felt either after a reduction in the activity of these physiological factors, or it may be the *Filament* action itself becomes sufficiently strong to overcome this stabilising influence. Both explanations are possible.

(2) Once *Filament* gene comes into action, its effect does not persist uninterrupted, throughout the succeeding stages. There is a break at the 8th batch, where once again, the *Filament* ovaries regain their normal activity and produce practically the same number of eggs as controls. This again, as in the previous case, indicates either a reappearance of the influence existing at the start, or a temporary deterioration of *Filament* action. The exact explanation is outside the scope of this investigation,

but when we see that both the temporary lapses of *Filament* action are associated with peak activities (when more than the normal batch-outputs of eggs are produced) of the ovaries, it looks probable, that the forces or influences at work that tend to make the ovaries act normally, are physiological factors—normally occurring in the flies at specific intervals—rather than any peculiarity of *Filament* action. This fact is of special interest, as it clearly shows, that even a decidedly deleterious effect as that of *Filament* can be successively overcome by forces other than genetical. An understanding of these forces should enable us to control egg production and get normal output of eggs even when genetical make-ups of the flies are working against it.

So far, we have dealt only with batch-outputs and the changes occurring therein, as batch follows batch—but what about the times taken by the various batches to elaborate the eggs and lay them? How do these behave in the two sets of flies? Unfortunately, with the counting intervals employed by us (12 hr.), extent of batch-duration cannot accurately be determined. Donald and Lamy (1936) who employed like intervals for egg counting have used the following method for finding batch-duration:

They take the intervals between the central points of successive "hollows" of the egg production curve, expressed in 12 hr units, to represent the batch interval or as they put it "the wave-length". This method is subject to serious error when employed on data gathered for a short period (here 15 days) as the "length" remains practically the same (3 units) throughout. The error introduced in our calculations, if we use this method here, would be of the order of ± 1 unit, i.e., 33 $\frac{1}{3}\%$. Hence, we have not attempted the analysis of wave-duration.

The other points now awaiting clarification are.—

- (1) Is the average egg production for the entire period of testing the same for *Filament* and *Control*?
- (2) How does the average rate of egg production as also,
- (3) The average rate of change in egg production, behave in the two cases?
- (4) Are the egg production curves of *Filament* and *Control* similar?

To answer these questions, Fisher's method of orthogonal Polynomial fitting was used. (The very same method was also employed in the analysis of the hatchability and fertility data that follow.) The results are given in Table IV.

TABLE IV

	A	B	C
Control	83 4808 ± 0 9862	-1.1871 ± 0 3062	-0.3172 ± 0.1047
Filament	72 8492 ± 0 7891	-1.9246 ± 0 2455	-0.1926 ± 0 0748
Difference	10 6316 ± 1.2637†	0 7375 ± 0 3925	-0.1246 ± 0 1627

† Highly significant Figure after \pm is S E

A, B and C are the respective coefficients that give us (1) average, (2) rate, (3) rate of change of egg production in the two cases. Comparison of the corresponding coefficients show that—

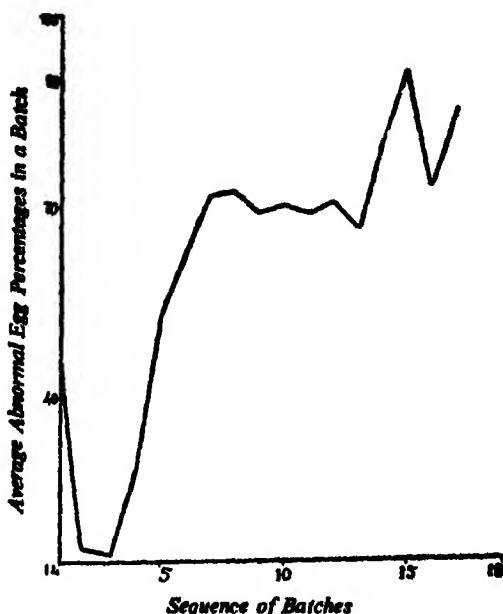
- (1) A of *Filament* is significantly different from the A of *Control*.
- (2) B of *Filament* is not significantly different from the B of *Control*.
- (3) C of *Filament* is not significantly different from the C of *Control*

This means that *Filament* flies on an average produce significantly lower number of eggs, whereas, as regards the other changes occurring in their mode of production and laying, they show practically no difference from similar changes occurring in the *Controls*. The trend of the egg production curve besides, is similar in the two cases, only *Filament* has throughout a lower level of production.

EFFECT OF AGE ON THE PRODUCTION OF ABNORMAL EGGS

As a count of the abnormalities occurring in the total eggs produced by the *Filament* flies had been separately kept, it was found possible to work out the effect of aging of the flies on the production of abnormal eggs. The results are brought out in Graph III. As can be seen from the graph, the abnormal egg percentage is fairly high in the first batch, it drops to a minimum in the succeeding two batches, and thereafter shows progressive increase, so that, after the fifth batch it remains at a consistently high level. The increase in the abnormal egg percentage after the third batch may be ascribed to the effect of age, but it is difficult to understand why after starting with fairly high percentages of abnormal eggs, the flies suddenly produce fewer abnormalities in the two immediately succeeding batches. Two things however are clear at this stage and they are.—

- (1) The first clear indication of *Filament* action on egg production is felt only after the second batch eggs have been produced and laid, i.e., roughly after two days from the start of egg-laying.



GRAPH III Graph showing the Variation in Abnormal Egg Percentage with Batch

(2) *Filament* action on egg shape and size on the contrary, is present from the start of egg production.

In explaining the delayed action of *Filament* in (1), we had suggested that probably the delay is due to the influence of beneficial physiological factors that come into play at certain stages and bring about peak production. It appears possible that these self-same forces may also be the cause of the lowered production of abnormal eggs, as curiously enough, the lowering of abnormal eggs takes place precisely at the same time as peak production by ovaries.

HATCHABILITY

Aging of females has considerable effect on the hatchability of the eggs produced by *Filament* flies. The eggs laid in the first few days are more fertile than the eggs laid subsequently. In order to get an estimate of the extent of this effect, eggs of 7 available *Filament* females and 17 *Controls* were tested for hatchability. The females had been kept separately and the eggs also tested separately; but since their individual records showed no significant variation, only the totals of the results are given below:

TABLE V

Comparison of the Effects of Age on the Hatchability of Eggs produced by Filament and Wild type Flies

Day	Filament			Control		
	Total eggs kept	No hatched	Hatchability in %	Total eggs kept	No hatched	Hatchability in %
1	245	195	79.58	723	673	93.08
2	251	222	88.45	399	365	91.48
3	351	288	82.05	1128	1026	90.96
4	333	241	72.37	610	502	82.30
5	287	134	46.69	386	353	91.50
6	325	188	57.85	837	686	81.50
7	433	87	20.09	790	648	82.03
8	252	33	13.10	363	336	92.56
9	96	10	10.42	129	126	97.67
10	59	1	1.70	360	301	83.61

Filament eggs start with a fairly high percentage of hatchability (100% hatchability is not possible due to the presence of sterile "No-filament" eggs), but it drops within the next ten days to almost zero. The fall is rapid, and the initial high figure is never regained. The controls on the contrary, start and maintain high hatchability throughout the same period. Comparison of the corresponding coefficients A, B and C is given in Table VI.

TABLE VI

	A	B	C
Filament	47.2310 ± 3.4031	-10.5312 ± 1.1848	-0.3985 ± 0.4683
Control	88.6690 ± 1.9098	-0.2710 ± 0.6649	0.2648 ± 0.2648
Difference	-41.4380 ± 3.9023†	-10.2602 ± 1.3586†	0.6633 ± 0.5360

† Highly significant. Figure after ± is S.E.

FERTILITY

Fertility is measured from the number of adult flies emerging from a given number of eggs expressed as a percentage of the eggs kept. In carrying out fertility tests, 3 parallel sets were run. In (1), 12 *Filament* females were mated to their brothers only. In (2), the same number of *Filament* females were mated to *Wild type* males. In (3), 15 *Wild type* females were mated to their brothers. The last mentioned served as Controls. Eggs of individual females were collected and incubated separately. Not more

than 50 eggs were put in any vial containing approximately 1" of *Drosophila* food. Care was taken to keep the eggs of a particular batch in separate tubes. In this way, figures were obtained that gave a sample of eggs kept from a batch and later on, the number of adult flies that emerged—from which, the batch-fertility could be calculated. The results are brought out in Table VII.

TABLE VII
Successive Batch Fertility in the Three Matings

Wave No.	Fertility in percentage		
	<i>Filament</i> ♀	<i>Wild type</i> ♀	<i>Filament</i> ♂
	<i>Filament</i> ♂ (Mating I)	<i>Wild type</i> ♂ (Control) (Mating II)	<i>Wild type</i> ♂ (Mating III)
1	58.20 (1007)	69.18 (1174)	53.42 (438)
2	50.75 (1068)	66.76 (1169)	52.42 (659)
3	43.05 (1060)	71.27 (1365)	72.94 (606)
4	35.67 (1054)	65.57 (1505)	68.45 (481)
5	20.17 (952)	62.79 (1179)	51.42 (457)
6	9.95 (954)	58.70 (1380)	22.60 (553)
7	4.35 (803)	58.74 (1275)	14.20 (507)
8	0.14 (792)	70.74 (1254)	7.62 (407)
9	0.36 (727)	74.30 (1217)	5.37 (298)
10	0.44 (792)	76.06 (1118)	1.59 (44)

The numbers in brackets give the total number of eggs incubated in each case.

As compared with the hatchability the fertility figures are decidedly lower which show that some of the eggs that hatch, fail to complete the life-cycle. Secondly, whereas the control fertility shows no appreciable change (within the period tested) the fertility in other two cases, drops down in later batches, so that it is practically nil at the end of the tenth wave. Further, the drop in fertility is more rapid in *Filament* flies fertilised by *Filament* males than when they are fertilised by *Wild type* males which means that some of the eggs that die if fertilised by *Filament* sperms, survive and reach adult stage—if fertilised by *Wild type* sperms. Besides, *Filament* eggs, when fertilised by *Wild type* sperms maintain a high fertility for a period of two more batches. As the fertility is nil after the tenth batch (irrespective of the male used), it is apparent that the action of the sperm is not able to check the total loss of fertility that occurs after the tenth batch. This point will be discussed later. The three sets of figures were analysed using the method previously mentioned and the corresponding coefficients so obtained were compared and tested for significance. Results are given below—

TABLE VIII

Comparison of the Successive Batch Fertilities of the 3 Matings

(i)	Mating No I	<i>V_s</i>	Mating No II
	Filament ♀		Wild type ♀
	Filament ♂		Wild type ♂
	A	B	C
I	22.3081 ± 1.1417	-7 2200 ± 0.3975	0.6443 ± 0.1571
II	66.6158 ± 4.1330	0 5301 ± 1.4377	0.5783 ± 0.5683
I-II	-44.3078 ± 4.2878†	-7 7501 ± 1.4916†	0.0660 ± 0.5896

(ii)	Mating No II	<i>V_s</i>	Mating No III
	Filament ♀		Wild type ♀
	Filament ♂		Wild type ♂
	A	B	C
I	22.3080 ± 1.1417	-7 2200 ± 0.3975	0.6443 ± 0.1571
III	35.0030 ± 4.3527	-7 9636 ± 1.5154	-0.6779 ± 0.3990
I-III	-12.6950 ± 4.4999†	0 7436 ± 1.5825	1.3222 ± 0.6191*

(iii)	Mating No III	<i>V_s</i>	Mating No II
	Filament ♀		Wild type ♀
	Control ♂		Wild type ♂
	A	B	C
III	35.0030 ± 4.3527	-7.9636 ± 1.5154	-0.6779 ± 0.3990
II	66.6158 ± 4.1330	0.5301 ± 1.4377	0.5783 ± 0.5683
III-II	-31.6128 ± 6.0023†	-8 4937 ± 2.0889†	-1.2562 ± 0.8257

* Significant † Highly significant Figure after ± is S E

The analysis shows.—

(1) *Filament* flies (whether mated to brothers or wild type males) have a decidedly lower fertility.

(2) Whereas, *Control* fertility shows no appreciable change throughout the 15-day test, *Filament* fertility (irrespective of the kind of male used) shows a highly significant drop with advancing age. No flies are obtained after the 10th batch. The drop in fertility besides, is too rapid and steep to be explained as an aging influence. It looks we are dealing here with an instance of "progressive abnormality" of the ovary.

(3) *Filament* females fertilised by *Wild type* males give a significantly higher fertility than when their own brothers were used for fertilization. Besides, in the former instance, the higher fertility is also maintained for an additional period of two more batches (4th to 5th). The only possible reason for this differential fertility, is the probability of the failure of the homozygous *Filament* individuals to reach adult stage. Most of the known dominant mutants are lethal when homozygous. It has therefore been found difficult to get pure stocks in their cases. In *Filament* too, though selective breeding has been going on for more than twenty generations, it has not been possible yet to get a pure stock. On the assumption that *Filament* too is lethal when homozygous, we should expect to get only $\frac{1}{2}$ of *Filament* by *Wild type* fertility, when males from *Filament* stock were used. The expected fertility works out to 26.25, whereas the actual observed figure is only 22.3. The difference between expected and observed fertility may in part be explained by the individual variation in the occurrence of sterile "No-filament" eggs, which would bring down the total fertility and also due to the random nature of sampling of flies selected for testing.

GENETICAL

In an attempt to get a homozygous stock, pair matings of tested *Filament* females with their brothers from same cultures were made. The resulting daughters from each vial were separately tested. Vials yielding a high percentage of *Filament* females were selected for further breeding. In spite of the fact that this type of selection was carried on for nearly 20 or more generations, no homozygous stock could be obtained. The percentage could never be increased to more than 60. Following is the result of a recent experiment in which flies from 57 cultures were tested.

No of cultures	Total flies examined	No Filament	% Filament
57	322	177	54.97

GENETIC CONSTITUTION OF THE NORMAL LAYERS IN FILAMENT STOCK

For this test, 18 normal layers (virgin) from *Filament* stock were selected and divided into 3 lots of 6 flies each. Only one *Wild type* male was introduced in each lot. Each lot flies were kept together in separate vials and removed to fresh ones every four days to avoid overcrowding of cultures. The F_1 females obtained from each lot were subjected to separate tests.

If normal layers from *Filament* stock are genetically normal, then no *Filament* flies are expected in F_1 . If on the contrary, they are normal because of the overlapping of *Filament* with *Wild type*, then at least a few of the F_1 flies will show the *Filament* character. The results given below show conclusively that normal layers are in fact, genetically too normal. *Filament* turns out to be a simple dominant.

Lot No	No of F_1 females tested	No of <i>Filament</i> flies
1	36	—
2	58	—
3	52	—

Location of Filament

Filament like *spheroidal*, offers no satisfactory means of distinguishing the genotype from a study of external appearance. The character becomes visible in females only from the kind of eggs they lay and in males from the kind of eggs laid by their daughters. To obtain information on segregation therefore, it was necessary to test a very large number of females individually in a short time. The method now in vogue for the collection of eggs was found to be unsatisfactory and hence the development of a new technique to meet our special requirements was found necessary.

Spencer (1937 a, b) has recently shown that *Drosophila* females can lay eggs through a silk mesh onto a suitable medium. His method consists in keeping the females in metal hose gas ferrules ($\frac{7}{8}$ " d. $\frac{1}{2}$ " h) covered at one end by silk bobinette, the opposite ends being closed by corks. The silk net is kept in contact with the medium and the females are thus allowed to lay eggs through them. A modification of Spencer's technique was employed and it served our purpose admirably. Instead of the metal gas ferrule, 2 gram pill boxes were used. The bottom of the box and lid were knocked off, which gave us two rings of different widths which slid over each other. The smaller ring served as a catch to keep the silk mesh tightly in position. Both rings were then soaked in melted paraffin wax to prevent absorption of moisture from media. The top, away from the mesh, was closed by an ordinary pill box lid, on the top of which appropriate letterings could be written. Bridges' food in large petri-dishes containing a fine suspension of fresh yeast served as a medium for laying. The above method was also employed in hatchability and other experiments where females had to be "tested".

Chromosome of Filament

In finding the chromosome of *Filament* the following scheme of work was adopted:—

(1) Pair matings of *Filament* flies were made in the first instance.

(2) The resulting females from each culture were tested separately. The culture giving the highest percentage of *Filament* flies was retained and the others discarded.

(3) 30 males from the selected culture were then mated to 30 females (virgins) from a "testor" stock containing the "markers" *vermillion* (*v*)—an eye colour; *purple* (*pr*)—also an eye colour, *tangled* (*tg*)—a wing vein disturbant and *arristopedia* (*arr*) which affects the arrista of the flies. This "testor" stock and before use the selected virgin females from them had been tested and found to contain no *Filament*.

(4) F_1 males from each culture were kept separately from the females. Both the vials along with original vial, were kept held together by means of elastic rubber bands

(5) After four or five days, vials containing the females were examined for *Filament* eggs.

(6) Only the vials yielding *Filament* eggs were retained for further study and the rest discarded. This precaution was necessary as certain of the P_1 males used may not be genetically *Filament*. Let these retained vials be called X-vials.

(7) 30 Males from each of the X-vials were backcrossed to virgin *v pr tg arr* females "tested" for normality. These 30 vials were kept together by elastic bands giving as many vial groups as the number of X-vials.

(8) F_2 from each group were collected *enmasse* and separated into different sub-groups, according to the combination of marker genes shown by them. At least 10 females belonging to each sub-group and therefore showing a given combination of marker genes, were tested. The summary of the data obtained is given below.

If all the F_2 females lay normal eggs, the obvious conclusion will be that *Filament* is sex-linked. If on the contrary, certain *Filament* flies are obtained, then *Filament* must be situated on one of the autosomes. The recombination data (Table IX) gives a clue.

TABLE IX

Genotype	Normal layers	Filament layers
v	57	36
v arr	73	
v pr	31	28
v tg	48	26
v arr pr	37	
v arr pr tg	45	
v pr tg	41	13
v pr arr	55	
Total	387	103

The reappearance of *Filament* at once shows that *Filament* is not sex-linked. The absence of recombination of *Filament* with *arristopedia* further shows that *Filament* gene is located on the same chromosome and belongs to the same group of mutants as *arristopedia*, i.e., II group.

SUMMARY

1. A complete description is given of a new egg mutant *Filament* (F) occurring in *Drosophila pseudo-obscura*
2. *Filament* condition becomes apparent only from the eggs laid by females. So far, no satisfactory means of ascertaining the genotype of the fly from its external morphology, has been found.
3. The main visible effect of *Filament* gene is on the two filaments. Only about 50% of the eggs laid by *Filament* flies are abnormal; others to all appearance are normal. Genetic tests however revealed, that they are genetically *Filament*. The observed variation in egg abnormality therefore is due to the poor "penetration" of the *Filament* gene.
4. The abnormal eggs fall into various categories according to the degree of abnormality shown. This depends on the "fusion" and "reduction" of filaments.
5. Variation is observed in the hatchability of abnormal eggs depending upon the category to which they belong. No-filament eggs for instance without exception, are sterile. As a rule, it is shown that more the departure from the normal shape, less is the hatchability.
6. Comparison of the widths and lengths of *Filament* and Wild type eggs show that the former are narrower and shorter than the latter. The differences are statistically significant.

7 Fecundity tests show that *Filament* flies on an average produce significantly lower number of eggs than *Wild type*. Detailed analysis further revealed that the action of *Filament* gene is shown only after a few days from start of laying. Besides, even after it comes into action, its action does not remain unmodified throughout later stages. The probable factors at work that determine egg production are discussed.

8 Hatchability tests show that *Filament* flies show a progressive steep drop with advancing age. After 15 days from start of laying, hatchability is nil for all practical purposes. The drop is found to be too rapid to be accounted for as a mere aging effect and it is suggested that probably a "progressive abnormality" of the ovary is responsible.

9 Fertility figures show exactly the same trend as hatchability figures. Besides, the fertility of the females mated to *Wild type* males is higher than that of *Filament* females mated to their brothers. This increase is found to be statistically significant. The increase however does not succeed in stopping the downward trend once started and is unable to stop the total infertility that occurs after 15 days from start of laying. An explanation is given for the observed difference in fertility when different males were used for fertilising the *Filament* females. It is suggested (and the fact is borne out by the failure to get a homozygous stock of *Filament* even after 20 generations of selective breeding) that *Filament* is lethal when homozygous.

10 A special technique for carrying out the "testing" of females is described. Genetic tests carried out to locate *Filament*, show that it is situated on the same group of linked genes as *arristopedia* (*arr*), i.e., II group.

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PRELIMINARY NOTE ON AUTOTETRAPLOIDY IN CAJANUS INDICUS SPRENG*

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Received December 18, 1944

AUTOTETRAPLOIDY was induced in *rahar* (*Cajanus indicus* Spreng) by the application of colchicine. The material used was a strain of *rahar* which had been maintained in the Economic Botanist's collection for over eight years. Previous experience with other plants had shown that for a satisfactory growth of the treated plants it was better to have a diploid root system. Therefore, the apical buds of seedlings were treated without the roots being affected in any manner. Success was obtained when 1 per cent colchicine was applied by the agar-colchicine method (Kumar and Abraham†). Compared with *mung* (*Phaseolous radiatus* Linn.) which became affected when 0.4 per cent. solution of the drug was used *rahar* showed effect only when 1.0 per cent. solution was applied. The affected buds were very slow in developing compared with the control but after a fortnight produced the first few leaves which were thickened and deformed. Leaves developed later although appeared normal in shape, were thicker, more hairy and darker in colour. In vegetative growth although the affected plants were slow at first, once they recovered from the initial setback, grew as fast as the controls. Unlike *rahar*, affected *mung* plants of the first colchicine generation could not get over the initial setback and continued to lag behind the control in growth to the very end. The necessity of a stronger solution required to induce polyploidy and its ability to recover from the early set back would show that *rahar* has the capacity to overcome the effect of colchicine either by producing some thing that neutralises the effect of the drug or effecting a quicker elimination of the drug from the meristematic region.

A large number of seedlings were treated, but only 36 were transplanted in the field. As the treatment was given in early October 1941, and the plants were transplanted in the cold months that followed, the plants failed to flower

* Read before the joint session of the National and Indian Academies of Sciences, held in Poona in December, 1944.

† Kumar, L. S. S., and Abraham, A., *J. Bombay University*, 1942, 11, 3, 30

that year. In the summer of 1942 all the plants were pruned down and during the second year of their growth all the control plants flowered profusely and produced a large number of pods. Of those suspected to be polyploids from external appearance a few shed all their flowers and did not set a single pod. Cytological examination of all the plants showed that two were definitely tetraploid and all the rest diploid. The two tetraploids (plant Nos. 27 and 36) did not set pods until March 1943, when a certain number of pods with a few well formed seeds in each were obtained.

Several workers have reported the striking difference observed in respect of plant characters between the colchicine induced polyploid and its diploid progenitor. In many cases, the plant as a whole and its various parts have exhibited gigantism. For this reason, the two tetraploid individuals were carefully compared with their diploid controls for some of the quantitative and qualitative characters.

The following is a comparative feature of the tetraploid and diploid for some of the characters examined.

I MORPHOLOGICAL AND ANATOMICAL

<i>(a) Quantitative Characters</i>	<i>Diploid</i>	<i>Tetraploid</i>
(i) Chromosome number $2n =$	22	44
(ii) Leaf size—length	11.2 cm	12 cm.
breadth	.43 cm	4.5 cm.
(iii) Pollen sterility	.. 9%	64% to 74%
(iv) Pollen size—diameter	.. 0.25 mm	0.21 mm.
(v) Chlorophyll content of leaf	{ There was no marked difference between the diploid and tetraploid	
(vi) Size of stomata		
(vii) Size of flower	Flower of the tetraploid are about $1\frac{1}{2}$ times as large as those of the diploid.	

(b) Qualitative Characters

- (i) Leaves of the tetraploid are somewhat darker green than those of the diploid. Transverse section of the leaves of the tetraploid compared with diploid showed no marked difference either in the thickness or internal anatomy.
- (ii) Tetraploid is later in flowering than the diploid.

- (iii) There is no pod formation during the greater part of the season and only a few pods are set towards the close of the season in the tetraploid. Diploid sets pod abundantly.
- (iv) In the tetraploid, the pods when set mostly contain one or two seeds only as compared to three or four in the diploid.
- (v) A good number of the tetraploid seeds are shrunken and shrivelled up and appear unhealthy while most diploid seeds are sound.
- (vi) The germination percentage of the diploid seed is about 90 while that of the tetraploid is about 25 per cent. Tetraploid seeds take about a week more to germinate than diploid seeds.

From the above it will be seen that the usual effect of tetraploidy resulting in *gigas* characters is not to be seen in *rahar* except for increased leaf and flower size.

II CYTOLOGICAL

Besides comparison of the morphological and anatomical features of the tetraploid and the diploid *rahar*, the behaviour of the chromosomes during nuclear division in pollen mother cells of the two types were compared as explained below.

(a) *Diploid*—Hundreds of P M C's of the diploid *rahar* were examined and invariably in all of them only 11 bivalents were found. Pairing and anaphasic separation were normal.

(b) *Autotetraploid*—A large number of P M C's of the tetraploid plants were examined and chromosome conjugations in 52 of them were analysed. The results are tabulated below.

From Table I it will be observed that there is a high degree of multivalent formation. There is not even one P M C. in which there are not some multivalents atleast. The average number of multivalents per cell is 4.88. The extreme case, when the 44 chromosomes are present as 11 tetravalents has been met with rather more frequently in this case than in similar auto-tetraploids in other species. In 6 out of 52 P.M.C.'s, such a state is present. Muntzing (1936) has classified the autopolyploids into two main categories. (a) Organisms, wherein all the chromosomes may form multivalents with a variable number of other conjugations in different cells and (b) Organisms wherein only a very low percentage of chromosomes form multivalents. Autotetraploid *Cajanus*, therefore, seems to belong to the first category.

TABLE I

Chromosome conjugation at M. I of Plant No. 27

Univalents	Bi	Tri	Tetra	Penta	No. of P. M. C.
1	9	7	1	-	1
1	9	3	4	-	1
1	19	3	2	-	1
1	14	5	-	-	1
1	5	7	3	..	2
1	6	5	3	..	2
1	14	1	3	..	1
1	20	1	-	..	2
1	17	3	1	-	1
1	14	3	-	-	1
2	6	10	1	-	1
2	8	6	2	-	1
2	15	3	-	-	1
3	7	5	2	-	1
3	12	3	2	-	1
3	16	3	-	-	1
3	8	3	4	-	1
2	14	2	2	-	1
1	8	4	7	-	4
1	14	6	11	-	1
1	9	2	2	-	1
1	7	4	6	-	1
1	4	4	7	-	1
2	2	4	6	-	1
10	10	4	6	-	2
10	6	4	6	-	1
13	6	6	-	-	1
11	6	2	1	-	2
17	6	2	-	-	1
14	2	2	1	-	1
12	6	2	-	-	2
6	6	2	2	-	1
15	3	2	-	1	1
8	6	2	1	..	1
3	2	2	6	-	1
2	..	10	11	-	6
22	278	143	111	1	52
Mean 0.81	5.34	2.73	2.18	0.2	

Fig. 1 and Figs. 3 to 6 show some of the chromosome groups of the tetraploid plant No. 27 while Fig. 2 is that of plant No. 27-6. Fig. 1 is that of a somatic plate from the root-tip showing 44 chromosomes. Figs. 2 and 3 represent respectively 22 bivalents and 11 tetra-valents at metaphase I in the pollen mother cells. Figs. 4, 5 and 6 show three different types of chromosome conjugation at metaphase I in the pollen mother cells.

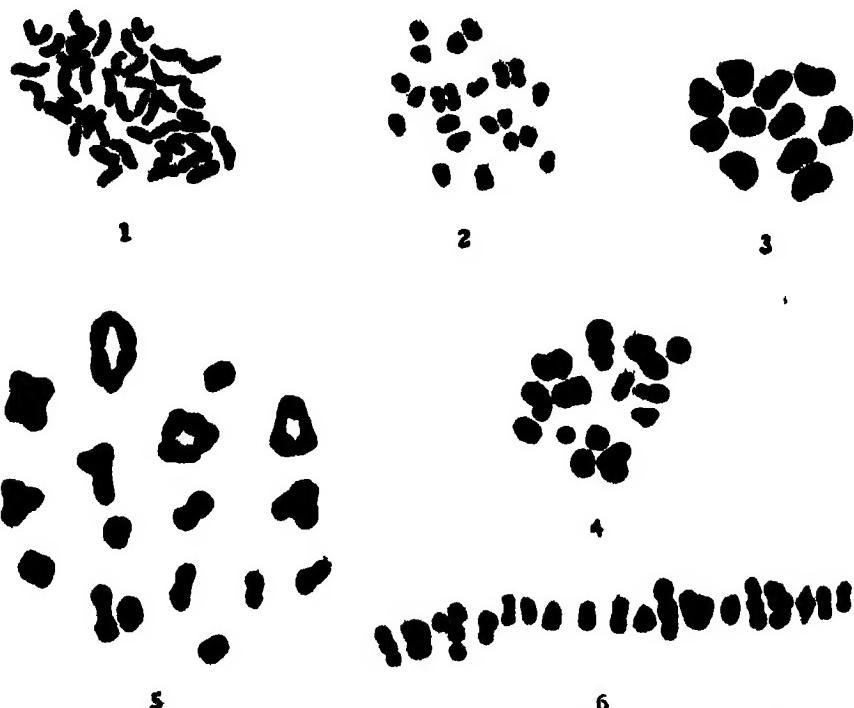


Fig 1 Somatic metaphase plate, $2n = 44 > 2500$ Fig 2 M I, 22 bivalents $\times 2500$
 Fig. 3. M I, 11 tetravalents $\times 2500$ Fig 4 M I, $4_n + 4_n + 6_n \times 2500$ Fig 5 M I,
 $10_n + 4_n + 3_n \times 2500$ Fig 6 M I, $11_n + 6_n + 1_n \times 2500$ (Side view, separated
 while drawing).

A few seeds from the tetraploid plant No. 27 were harvested for raising its progeny. These seeds gave very low percentage of germination and were very slow in developing. Out of a few seeds germinated one grew to maturity. This plant No. 27-6 showed large-sized, darkened leaves and bore flowers which were distinctly larger than those of the ordinary diploid plant. The behaviour of the chromosomes at meiosis of this plant was compared with that of its parent already given in Table I.

The chromosome conjugation in 27-6 is essentially similar to that in 27. The maximum association into 11 tetravalents, is not seen. This may be due to the smaller number of pollen mother cells examined. One instance of the association of the 44 chromosomes into 22 bivalents is also seen (Fig. 2).

TABLE II

*Chromosome Conjugation at Metaphase I in *Cajanus indicus*
27-6 (Progeny of the original tetraploid *Cajanus* 27)*

Univalents	Bi	Tri	Tetra	Penta	No. of P.M.C.
1	16	1	2		1
	2	1	9		2
2	16	2		.	2
3	14	3	1		2
3	15	1	2		1
4	17	2			1
5	15	3		.	1
	16	.	3		1
	14	4	1		1
	16	4	.		2
	3	2	8		1
	9	6	2	..	1
	8		7		1
	19	2		.	1
	22				1
	11	6	1		1
	13	2	2	.	1
	14	4	1	..	1
	2		10	.	1
19	244	43	50	Nil	22
Mean 0.72	11.1	2.0	2.3	Nil	

SUMMARY

Autotetraploidy was induced in *rahar* by the application of colchicine. The tetraploid progeny was compared with the diploid parent for various qualitative and quantitative characters. The results show that *gigas* characters are not present in the tetraploid progeny, except for increased leaf and flower size. A study of meiosis in the tetraploid plant revealed a high degree of multivalent formation resulting in partial sterility. A daughter tetraploid plant was raised from this tetraploid and a study of meiosis in the daughter plant revealed its similarity to that in the mother.

AN UNUSUAL CASE OF A GRANULOMATOUS POLYPOSIS OF THE LOWER ILEUM

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Received December 15, 1944

(Communicated by Diwan Bahadur Dr K R Ramanathan, F.A.Sc.)

A PIECE of the small intestine showing polypi was sent to me for opinion by Dr. H S. Mehta, at the time Hon'ble Surgeon, Sassoon Hospital, Poona, presently, Police Surgeon, Bombay. The history so far as could be obtained was as follows —

The patient, a Hindu male, aged 25 years, a fitter by occupation, was admitted into the emergency section of the Sassoon Hospital, Poona, for colicky pains in the belly. These pains were being experienced intermittently for about two months prior to admission. There was history of passage of blood and mucus per rectum. Latterly the pains had become very severe. A fortnight before admission he had experienced the first severe attack with passage of blood and slime in the stools. He had been treated for dysentery by some practitioners without relief. The last attack proved unbearable and compelled him to seek admission into Hospital on 18th September 1934.

On admission the patient was found in a collapsed state. The pain was 'agonising'. On examination, a lump was found in the right hypochondrium and a laparotomy revealed an entero-colic (ileo-caecal) intussusception. This was reduced with difficulty but on palpation of its terminal portion, represented by the lowermost foot of the ileum, a number of nodules were felt. This piece of the gut was, therefore, resected and an ileocolostomy was performed. He died from shock 48 hours after the operation. No post-mortem examination was allowed by the relatives.

So far, a summary culled from Dr. Mehta's notes, so kindly placed at my disposal. The following abstract is taken from my own records concerning the naked eye appearance of the piece of intestine sent to the laboratory.—

The piece of the ileum sent is about 12 inches in length. The wall of the gut is irregularly thickened and the *valvulae conniventes* partly or wholly obliterated. There are four large polypi in various parts of this segment.

The accompanying photograph shows the intestine opened along the anti-mesenteric line (Fig. 1). Two of the polypi will thus be seen to be along the attachment of the mesentery, while the other two are not so situated. Three of the polypi are sessile and two are bilobed. One is pedunculated and almond-shaped. One of the bilobed polypi has its component parts globular in shape while the other has two finger-shaped divisions. The fourth is more irregular in shape but is more inclined to the globular for descriptive purposes. In addition to the above polypi there are smaller elevations. They are about the size of a mustard seed or smaller. Some of these show umbilication. These smaller projections may be the starting points of fresh polypi. One of the larger polypi forms a definite protuberance on the peritoneal aspect of the gut.

HISTOLOGICAL STRUCTURE

The following description applies principally to the largest or almond-shaped polypus which has been more thoroughly studied. It may, however, be stated that pieces of two other polypi have been sectioned though the section did not extend across the gut wall. Such a measure was deemed necessary to save the gross specimen for the museum.—

Under the low power it can be discerned that that portion constituting the apex of the growth, namely, the part lying free in the lumen of the gut, does not show any mucous membrane. On the other hand, the free portion of the globular polypus shows a mass of fibrin covering it. In one of the pieces this fibrin shows vascularisation. The tumour mass is found to extend in the circular muscular layer and projections, either finger-like or round, are seen displacing the muscle fibres and apparently pushing them aside (Fig. 3). In places the cells are found more scattered in the surrounding tissues. The blood vessels in the mucous membrane, where the latter exists, and in the submucosa are distended with blood. Engorged blood vessels of variable size are in evidence in particular in the subepithelial layer of the villi. In this connection, however, the recent reduction of an intussusception has to be borne in mind. Fibroblasts, endothelial cells and lymphoid cells are present in variable numbers, while large epithelioid cells with poorly staining nuclei and containing one or two nucleoli, are seen. These last cells are more numerous in some places than in others and in some of these cells the nuclear membrane is unusually well defined. One is also struck with the large number of eosinophiles (Fig. 5) and a considerable number of plasma cells present. I have failed to find any typical Sternberg-Reid lymphadenoma cells. Specimens stained with van-Gieson's stain show fibrous tissue which is most marked at the edge of the

lobulations of the cellular mass (Figs 6 and 7) Reticulum fibres are also present in sections treated with silver As regards lymphoid tissue, one finds here and there islets that may have been parts of larger masses of this tissue and in some sections one cannot resist the temptation of inferring that the surrounding cells have crept into this tissue and replaced it to a greater or lesser extent (Fig 8) From the history given, one is led to believe that there might have been attacks of intussusception prior to the last fatal instance recorded. One is not therefore surprised to find blood pigment in the midst of the tumour cells. In some places one also observes some large yellowish coloured cells with a small nucleus (Fig 9) These, to my mind, are phagocytic cells that have taken up blood pigment As one would expect, they are not present in areas of fresh haemorrhage where intact red cells are observable in sections

DISCUSSION

On naked eye examination, I was inclined to consider the case as one of lymphosarcoma Under very low magnification one felt that the tumour was a neoplasm and that it possessed invasive properties One cannot, of course, dispose off the latter possibility but the structure described is definitely that of a granuloma The enlarged glands in the mesentery of which, very unfortunately, I could not secure a specimen, led me to the belief that the polypi were only a part of a lymphadenomatous affection of the abdominal lymphoid tissue As the histological picture was equivocal, I referred the sections with a photograph of the gross specimen to various well-known authorities in the United Kingdom and to a leading Pathologist in Germany. The following are extracts from some of the views expressed:—

- 1 "I concur in your diagnosis of Hodgkin's Disease."
- 2 " . . . The case is most probably one of Hodgkin's Disease but one cannot be certain . . ."
- 3 "I cannot accept the two sections as demonstrating lymphogranuloma. . . What exactly the condition is I do not know. . . I did not find anything like parasitic eggs."
4. "I must at the start make it clear that without a report of a full post-mortem examination, I am not able to give a definite opinion "

It will thus be seen that the above views are divergent. In this connection, however, the following extract from Ewing seems of interest.—

"Gastro-intestinal Hodgkin's granuloma is an ill-defined condition, difficult to separate from tuberculosis on one hand and lymphosarcoma on

the other (Stoerk, Wells, Symmonds). . . There remains a considerable group of locally destructive hyperplastic lesions, localised in any portion of the gastro-intestinal tract, specially in the stomach, ileum and cæcum, in which the structure is distinctly granulomatous and in which tubercle bacilli are missing I have studied several cases of this type with local ulceration and extensive swelling of regional lymph-nodes, in which the lesions resembled Hodgkin's granuloma "

REFERENCE

Ewing

Neoplastic Diseases, 1941.

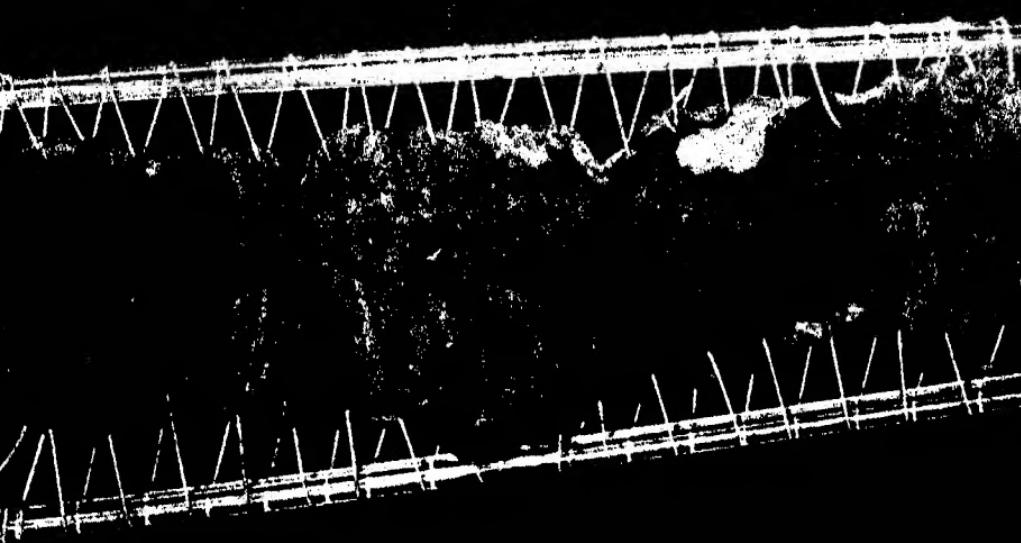


FIG. 1. The gross appearance of the piece of intestine.



circular muscle fibres by insinuation
in cellular mass.
t - polypus. m - circular fibres of intestine.



FIG. 5. a - Eosinophiles.



general view of the cells.



FIG. 6. Fibrous tissue is well marked between the



Fibrous tissue in the cellular mass.

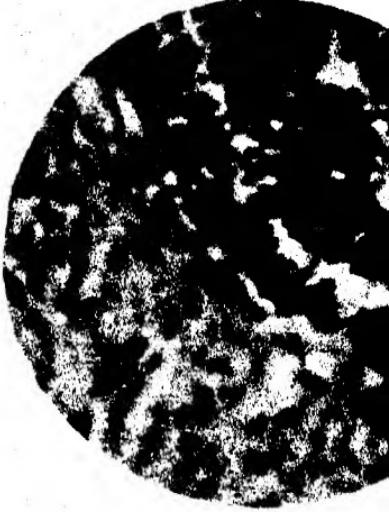
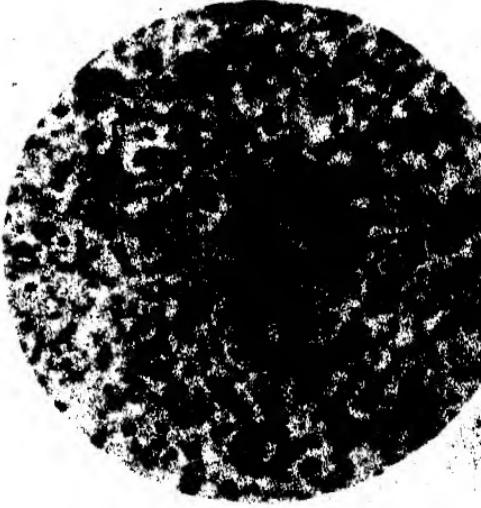


FIG. 8. Apparent replacement of lym-



A STUDY OF THE CORRELATION BETWEEN THE HABITS AND THE DEVELOPMENT OF THE ACOUSTIC CENTRE IN THE BRAINS OF CERTAIN AIR-BREATHING FISHES OF INDIA

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INTRODUCTION

IN previous communications (Bhimachar, 1935, 1937) it was pointed out that the surface feeding cyprinoids such as the species of *Rasbora*, *Chela*, *Danio* and *Esomus* and Cyprinodonts—*Gambusia*, *Lebiasina*, *Haplochilus* and *Panchax* possess a remarkably well-developed central acoustic lobe in their brain. It was argued that the better development of the acoustic centre in these forms over other fishes which habitually live under water at the ground level, is due to the fact that these fishes are constantly exposed to the external sounds at the surface of water and thus come to possess a keen sense of hearing.

There is another group of fishes in India the members of which, though belong to diversified families and orders, have a common feature about them, namely the air-breathing habit. It is well known that they come to the surface of water periodically to gulp in air at frequent intervals or are stranded outside water on mud flats, which they can resist for shorter or longer periods depending upon the extent of their air-breathing habit. Though the purpose for which these fishes are exposed to the external atmosphere is different to the one in the case of the surface feeding cyprinoids and cyprinodonts it was considered worthwhile to see whether they also possess a pronounced acoustic centre in their brains. Detailed accounts of the bionomics of the various air-breathing fishes are found in the works of Das (1927, 1936, 1940) and Hora (1935).

MATERIAL AND METHOD

The brains of the following fishes have been examined.—

		Family
<i>Anabas scandens</i> (Deld.)	.	Anabantidae
<i>Ophicephalus striatus</i> Bloch,	..	Ophicephalidae

	Family
<i>Clarias batrachus</i> (Linn.)	Clariidae
<i>Heteropneustes fossilis</i> (Bloch) }	Cobitidae
<i>Lepidocephalus thermalis</i> (C.V.)	Gobiidae
<i>Periophthalmus koelreuteri</i> (Pall) }	Amphipnoidae
<i>Pseudapocryptes lanceolatus</i> (Bl & Sch) }	
<i>Amphipnous cuchia</i> (Ham. Buch)	

One set of brains was fixed in Bouin's fluid, microtome sections taken and the sections stained in iron haematoxylin for nuclear study. Another set was fixed in 5% potassium bichromate solution and the sections were stained by Auriens Kapper's modified method of Weigert Pal staining for the study of the fibre tracts.

CENTRAL ACOUSTIC LOBE

Just as in the surface feeding fishes, the acoustic centre in the brain of the 'climbing perch'—*Anabas scandens*—is a distinct lobe consisting of a mass of rounded grey cells. Fig 1 represents the transection of the medulla oblongata in the region of the acoustic lobe. The acoustic lobe

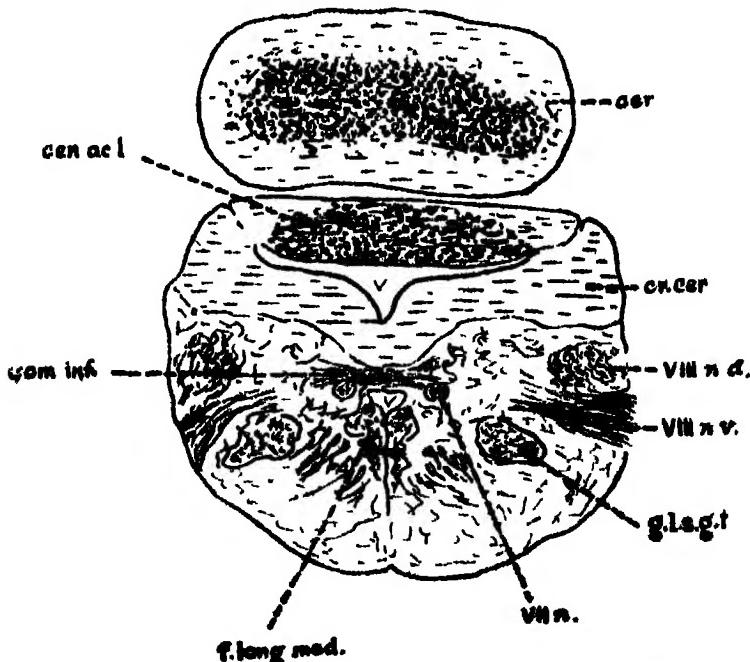


FIG 1 Cross-section of the medulla oblongata of *Anabas scandens* showing the central acoustic lobe. $\times 30$

is a spindle-shaped structure disposed transversely between the medulla oblongata and the cerebellum. The two ends are supported by the crista cerebelli of either side. The grey of this centre consists of a large number of deeply staining rounded cells interspersed between the layers of the stratum moleculare. In this region the crista cerebelli of either side are continuous with each other in the middle region with the result the fourth ventricle is divided into upper and lower cavities. A few sections rostralwards the caudal ends of the acoustico-lateral tubercles make their appearance and the molecular layer of the cerebellum becomes fused with the molecular layer of the acoustic lobe. The grey cell mass of the acoustic lobe becomes divided into two groups, one on either side leaving a cell-less area in the middle. Such a group of cells near the lateral recess of the ventricle in Teleosts is termed 'auricle' by Holmgren and Van der Horst (Pearson, 1936). More rostrally the acoustic nuclei become thinned out passing communicating fibres laterally to the respective acoustico-lateral tubercles, which if traced backwards can be seen joining the dorsal root of the VIII nerve. This region marks the anterior limit of the acoustic centre.

The structure of the central acoustic lobe in *Ophicephalus striatus* is, more or less, the same as in *Anabas* and hence a detailed description of it is considered unnecessary. In the Weigert Pal material it is seen that a portion of the dorsal root of the VIII nerve which is regarded as the acoustic branch is seen entering the acoustico-lateral tubercle and finally into the 'auricle', which, as has already been pointed out, is only the antero-lateral end of the acoustic lobe. Further it has been possible to study the development of the central acoustic lobe in the early larval stages of *Ophicephalus punctatus*. Fig. 2 represents the transection of the head of 7 mm. larva in the region of the central acoustic lobe. It is clearly seen that the cells of the central acoustic lobe are continuous over the region of the crista cerebellaris with similar granular cells of the acoustico-lateral area which indicates that the cells of the central acoustic lobe are derived from the acoustico-lateral grey.

With the development of the forwardly directed cerebellum in Siluroid fishes the disposition of the central acoustic lobe becomes slightly different as in *Heteropnusites fossilis* and *Clarias batrachus*. Fig. 3 represents the transection of the brain of *Heteropnusites* in the region of the central acoustic lobe. The acoustic nucleus is a wide arched lobe over the ventricle about the region of anterior end of the facial lobes. It is supported on either side by the crista cerebelli. About a few sections rostrally the grey mass of the central acoustic lobe becomes reduced and finally disappears before the appearance in the sections of the stratum granulosum of the cerebellum.

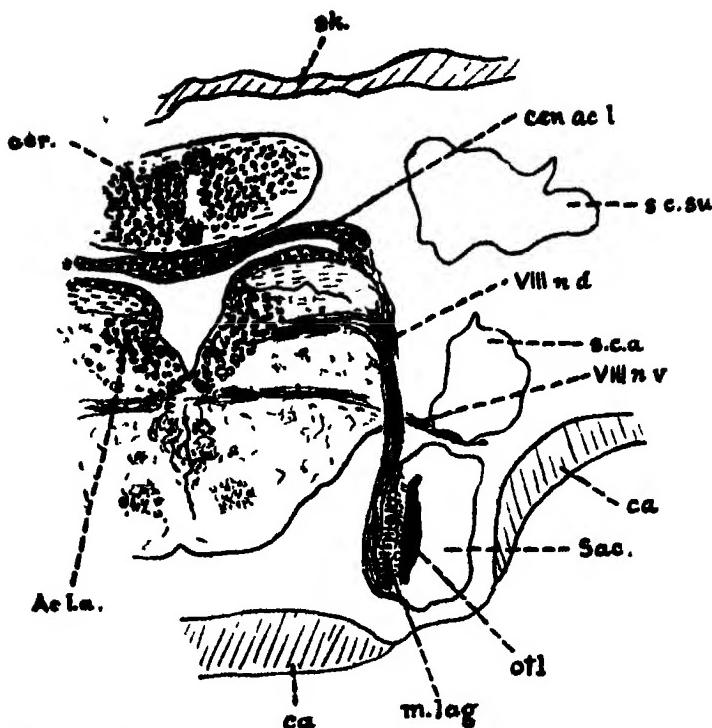


FIG 2 Cross-section of the head of 7 mm larva of *Ophicephalus punctatus* showing the central acoustic lobe and the dorsal root of the VIII nerve $\times 129$

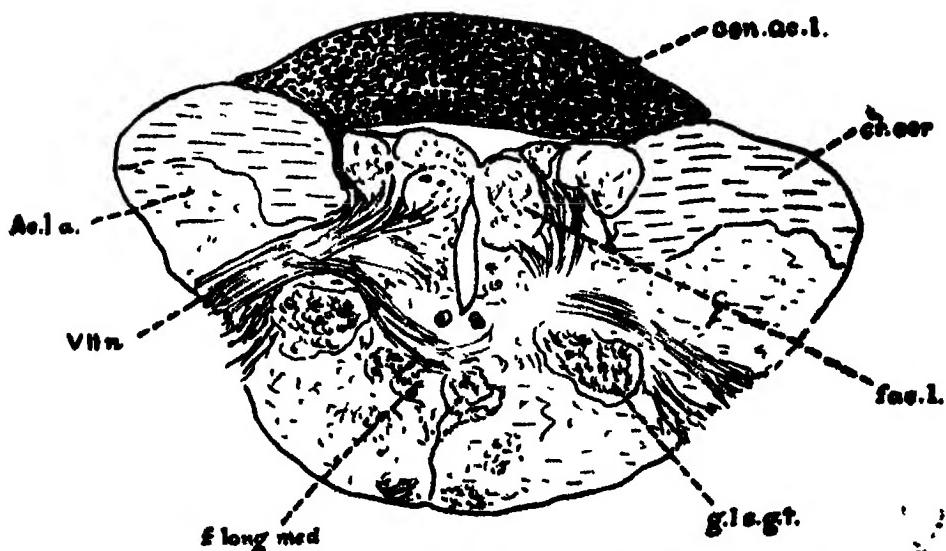


FIG 3 Cross-section of the medulla oblongata of *Heteropneustes fossilis* showing the central acoustic lobe $\times 30$

At the caudalmost end of the central acoustic lobe can be seen fibre tracts connecting this nucleus with the medial nucleus of the acoustico-lateral area.

The occurrence of a prominent central acoustic lobe has been noticed in *Lepidocephalus* (Bhimachar, 1935, Fig 3). In the other loaches—*Nemachilus* and *Nemachilichthys*—the acoustic centre is either absent or very feebly developed. The loaches grope and grub for food and live habitually at the ground level, with the result the sense of hearing is poorly developed in them. It is significant that *Lepidocephalus* possesses a well-defined acoustic lobe and this is due to its habit of coming to the surface of water at frequent intervals to gulp in air.

The central acoustic lobe in the Gobioids—*Periophthalmus* and *Pseudapocryptes*, is not as prominent as in other forms. The topographical relations of this lobe with other structures of the brain is the same as in other Teleosts. A definite fibre connection is noticed in both the fishes between the acoustic centre and the medial nucleus of the acoustico-lateral area (Fig 4). According to Pearson (1936 b) the medial nucleus receives the fibres from the dorsal root of the VIII nerve.

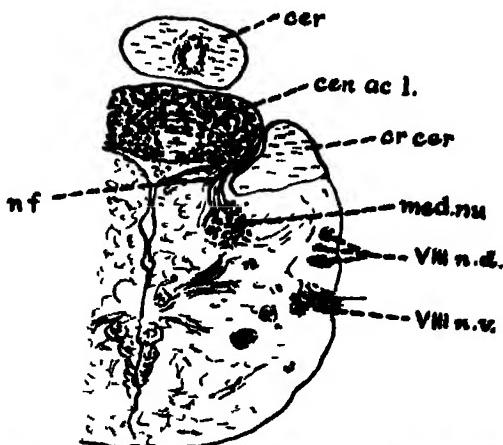


FIG 4 Cross-section of the medulla oblongata of *Pseudapocryptes lanceolatus* showing the nerve fibres passing from the central acoustic lobe to the medial nucleus of the acoustico-lateral area $\times 50$

The cells of the acoustic nucleus in *Amphipnous* are not comparatively numerous. But a remarkable feature here is that these cells are continuous with the cells of a nucleus situated at the dorso-lateral corner of the medulla oblongata and which, more or less, corresponds to the 'cochlear' nucleus (Larsell, 1934) of Amphibians—*Rana pipiens* and *Hyla regilla*. Further it is clearly seen that the dorsal fibres of the dorsal root

of the VIII nerve coming from sacculus and lagena of the ear which are regarded as organs of hearing in fishes (Frisch, 1936) terminate in the cells of this nucleus (Fig. 5)

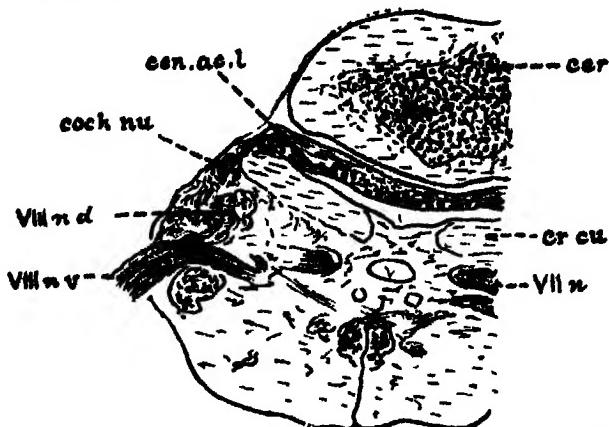


FIG. 5 Cross-section of the medulla oblongata of *Amphilophus cuchia* showing nerve fibres passing from the central acoustic lobe $\times 30$

Thus it is seen that the air-breathing fishes possess a pronounced development of the acoustic centre in their brains as in the case of surface feeding fishes. The reason attributed to this is that they are exposed constantly to the outside atmosphere, resulting in the better development of the sense of hearing. The elaboration of the peripheral sense organ has led to the greater differentiation of the corresponding brain centre namely the central acoustic lobe. While the primary inducement to come to the surface of water in the case of the cyprinoids and cyprinodonts is the problem of food which they get near the water surface in the form of abundant insect fauna and rich planktonic organisms, that in the case of air-breathing fishes is actually due to the air-breathing habit and other ecological factors prevailing in tropical waters.

HOMOLOGY OF THE CENTRAL ACOUSTIC LOBE

In spite of the fact that the presence of this nucleus is a constant and prominent feature of the brains of the Teleostean fishes, the available information on this in previous works is very vague. Kappers, Huber and Crosby (1936) while describing the cerebellum of Ganoids and Teleosts state "In certain regions the cells of the granular layer are grouped in such a way as to suggest a type of cerebellar nuclei. Such a group is found in the posterior part of the body of the cerebellum.... This group may correspond with the nucleus subcerebellosus of Ramóny Cajal's account...."

It is homologous with the stratum granulosum pars ventralis of Pearson (1936 a & b) He observes "The stratum granulosum in Teleosts, as in Amia, is divisible into two layers, a pars ventralis and a pars principalis. . . The stratum granulosum pars ventralis probably corresponds to the 'string of granular cells' figured by Palmgren and to the subcerebellar granular layer described by Van der Horst and Ariens Kappers . ." Evidently referring to this nucleus Kappers rightly remarks that in *Gadus* the tubercula acoustica fuse dorsally over the ventricle and there is a strong acoustic commissura in this area of fusion (Herrick, 1908) That this is a nucleus connected with the sense of hearing in fishes was first pointed out by Evans (1932) A study of this nucleus in a large number of Teleostean fishes (Evans, 1932, 1937 and Bhimachar, 1935, 1937) clearly indicates that it definitely belongs to the acoustico-lateral line system.

The contention that it is a part of the cerebellar nucleus appears to be untenable. The cells of this nucleus do not coalesce with the cerebellar grey and there is definitely no fibre connection between these two nuclei On the other hand, there are fibre connections between this nucleus and the acoustico-lateral tubercles Further the 'auricles' (antero-lateral tips of the central acoustic lobe) are seen connected by fibres with the medial nucleus of the acoustico-lateral area as in the Gobioids and they are directly connected with the fibres of the dorsal root of the VIII nerve as in *Amphipnous* and *Ophicephalus* Moreover during early development as in *Ophicephalus* it is seen that the granular cells of this nucleus are continuous with the granular cells of the acoustico-lateral area. It is thus clear that this centre though lies ventral to the cerebellum and partly embedded in the cerebellar molecular layer, is actually a part of the acoustico-lateral line system and therefore a part of the medulla oblongata

I am highly indebted to Professor A Subba Rau, D.Sc (Lond), F.R.M.S., for helpful suggestions and to my colleague Mr. P A Ramakrishna Iyer, M.Sc, for technical assistance in connection with this investigation

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ABBREVIATIONS

<i>Ac l.a</i>	. Acoustico-lateral area	<i>med.ru</i>	Medial nucleus
<i>Ca</i>	Cartilage	<i>n.fib</i>	Nerve fibres.
<i>Cent.ac l</i>	Central acoustic lobe	<i>Ost.</i>	Otolith
<i>Cer</i>	Cerebellum	<i>Sac</i>	Sacculus
<i>Coch nu</i>	Cochlear nucleus	<i>Sk</i>	Skin
<i>Com inf</i>	Commissura infima	<i>S.C.A</i>	Anterior semi-circular canal
<i>Cr cer</i>	Crista cerebelli	<i>S.C.Su</i>	Superior semicircular canal
<i>F lon med</i>	Fasciculus longitudinalis medialis.	<i>V</i>	Ventricle
<i>Fac l</i>	Facial lobe	<i>VII l</i>	Facial lobe.
<i>g.l.s.g.t.</i>	Great longitudinal secondary gustatory tract	<i>VII n</i>	VII nerve
<i>m.c.</i>	Methuner's cell	<i>VIII n.d</i>	VIII nerve
<i>m.lag</i>	Macula lagena	<i>VIII n.v</i>	Dorsal root of the VIII nerve Ventral root of the VIII nerve.

OBSERVATIONS ON THE CORRELATION BETWEEN THE SURFACE LIVING HABIT AND THE STRUCTURE OF THE BRAIN OF THE FRESHWATER GREY-MULLET, *MUGIL CORSULA* HAMILTON

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INTRODUCTION

THE study of the correlation of habits of fishes with their anatomical structure is one of the most fascinating subjects in Zoology. This gives a great clue to the structural changes occurring in the animal due to adaptation to any particular environment. The purpose of the present communication is to record a few observations on how far the pattern of the brain structure of *Mugil corsula* Hamilton reflects the general habit of the fish.

Studying the structure of the brain of certain cyprinoid fishes with reference to their feeding habits Evans (1931) and Bhimachar (1935) have classed them into three different groups. The habits and the brain structure of the Grey Mullet, conform, more or less, with the surface feeding cyprinoids. It may be stated that this paper does not attempt to give a detailed account of the brain structure but merely points out the important behaviour patterns as expressed by the habit of the fish.

The investigation of this problem was undertaken at the suggestion of Rai Bahadur Dr S L Hora, Director of Fisheries, Bengal, to whom the author is much indebted. The Bouin fixed material was sent by Dr. Hora. The brain sections are stained with iron haematoxylin.

SURFACE LIVING HABIT

Mugil corsula lives at the surface of water in ponds and rivers of the Gangetic provinces. To describe the surface living habit of this fish I cannot do better than to quote extracts from Dr. Hora's paper on the biology of *Mugil corsula* Hamilton, with observations on the probable mode of origin of aerial vision in fishes (1938). He states —

" *M. corsula* has the remarkable habit of swimming with its eyes above the surface of water. When the fish is swimming at the surface, the eyes, a portion of the head and the anterior part of the body are entirely out of water, the rest of the body is obliquely inclined to the surface of

water. As the fish progresses, ripples of the displaced water are found at the sides of the head . . Thus the fish moves through the water very gracefully and occasionally ducks its head below the surface presumably to keep the eyes moist. Its eyes are wholly out of water and are no doubt adapted mostly for an aerial vision. The stomach contents of several adult specimens contained nothing but algae and a few insects and young molluscs entangled among plants . When there are swarms of insects, *M. corsula* feeds voraciously on insects The stomach contents of small specimens from 6 to 8 cm in length were found to consist mostly of large number of copepods and sometimes small insects The almost toothless jaws, the presence of a symphisial knob and convoluted alimentary canal indicate that the observed feeding habits of *M. corsula* correspond with those of the 'Carp-minnows' Though the mouth of *M. corsula* is situated on the ventral surface of the head, when the eyes and a part of the head are out of water, its position becomes almost anterior and the gape becomes obliquely directed upwards and forwards, as is usually the case with the fishes that feed near the surface On the approach of an individual the fish dives under water with great agility but does not stay there for long and comes up to the surface at a short distance from its original position "

While describing this species Day (1889) observes "These fish swim with their eyes just above the surface of water, giving the appearance of a number of tadpoles Immediately they are disturbed they dive down with great rapidity"

SENSE ORGANS

It would be necessary to give a brief account of the sensory organs of the fish as they give a clue to (i) the habit of the fish and (ii) the extent of the development of their respective brain centres and connections The eyes are large and the visual sense is well developed. As the head of the fish projects outside the water for long periods, the eyes are adapted for aerial vision also Just as in the case of the surface feeding cyprinoids and cyprinodonts the sense of hearing is acute The lateral line sense organ of the trunk is absent. The gustatory organs namely the taste buds and the terminal buds are highly atrophied The barbels which bear large number of terminal buds, as found in cyprinoids and siluroids, are absent. The general tactile sense appears to be well developed

GROSS MORPHOLOGY OF THE BRAIN

The brain of *Mugil corsula* is relatively well developed. The medulla oblongata is narrow indicating hypertrophied condition of the visceral

centres such as the vagal, glassopharyngeal and the facial lobes. The acousticolateral tubercles, cerebellum, optic lobes and the forebrain are very well developed. Fig. 1 is intended to show how the brains of two

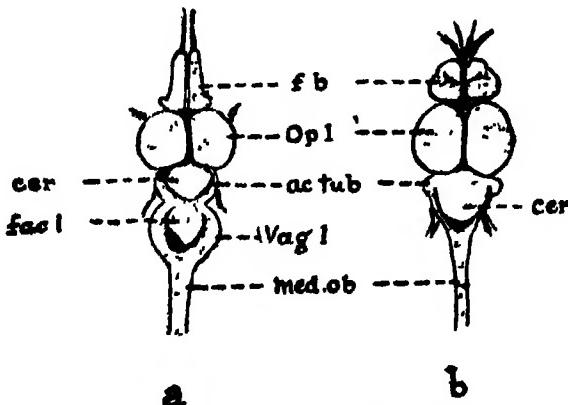


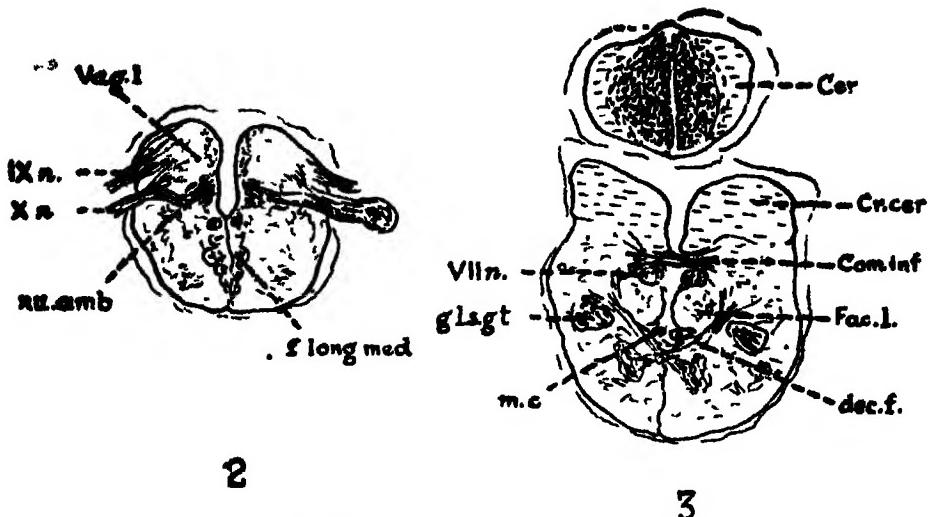
Fig. Dorsal view of the brain of (a) *Nemachilus beauforti*, (b) *Mugil corsule*

fishes with different habits—*Mugil* which lives at the surface of water and feeds mostly by sight and *Nemachilus* which lives at the ground and feeds by groping and grubbing, differ in their general structure

MICROSCOPIC ANATOMY OF THE BRAIN

The brain structure is examined from behind forwards. Fig 2 represents transection of the medulla oblongata in the region of the vagal lobes. Two small hypertrophied vagal lobes can be seen, one on either side of the ventricle. A few sections caudally the lobes just fuse with each other over the ventricle. The nucleus ambiguus (motor vagus nucleus) consists of large spindle-shaped cells and lies ventromesial to the visceral nucleus. There are decussating fibres passing down the vagal lobes. At the rostral end the two vagal lobes are connected with each other by commissura infima. The brain in this region is narrow. The nerve roots of the IX and the X nerves can be seen entering the brain. The cerebellum is cut in transection at the level of the facial lobes as represented in Fig 3. The facial lobes are comparatively very small and remain independent instead of fusing with each other as in many cyprinoids. The motor sensory column or the V lobe which is the brain centre for tactile sensation is prominently developed. Thick bundles of descending fibres from the V lobe are noticed. The secondary gustatory tracts are feebly developed.

About the rostral end of the medulla oblongata is seen a spindle-shaped mass of rounded deeply stained cells between the somatic sensory



Figs 2-3.—Fig. 2 Cross-section through the medulla oblongata of *Mugil corsicus* showing the vagal lobes $\times 30$. Fig. 3 Cross-section through the medulla oblongata of *Mugil corsicus* in the region of the facial lobes $\times 30$

column and the cerebellum which is designated by Evans (1932) as the central acoustic lobe. The central acoustic lobe contains a mass of grey composed mostly of rounded cells taking deep haematoxylin stain just as the cells of the stratum granulosum of the cerebellum. As the sections are examined rostrally, in the region where the acousticolateral tubercles appear the molecular layer of this lobe fuses with the molecular layer of the cerebellum and the rounded grey cells disappear in the middle region, leaving two masses of cells one on either side near the lateral recess of the ventricle. Communicating fibres from these cell masses can be seen passing laterally to the respective acousticolateral tubercles. A few sections rostralwards even these cells disappear. Just before the disappearance of this cell mass a branch of the VIII nerve can be seen entering this nucleus. The fact that these cells neither join the cell mass of the stratum granulosum of the cerebellum nor send any fibres to that area clearly indicates that it is not, in any sense, a part of the cerebellum.

The acousticolateral tubercles receive nerve fibres from the internal ear and the lateral line sense organs. The caudal ends of these tubercles are seen about the region of the commissura infima. They gradually increase in size rostrally and coalesce with the cerebellar grey at their anterior extremity. The cerebellum which is the brain centre for the static and the muscular tone is well developed.

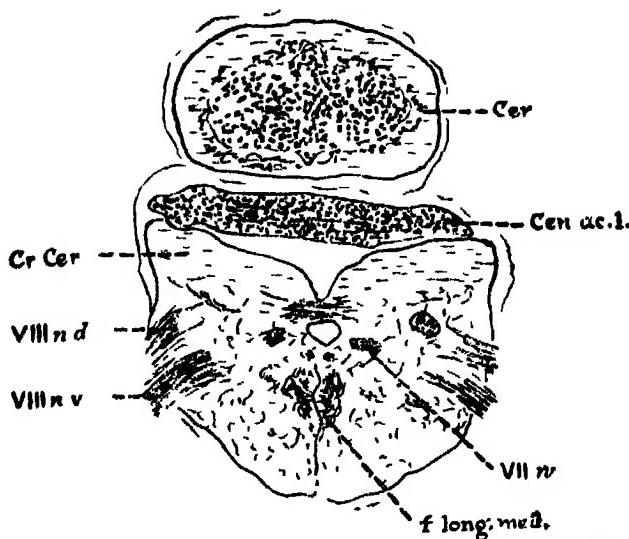


FIG. 4 Cross-section of the medulla oblongata of *Mugil corsula* showing the central acoustic lobe. $\times 30$

Fig. 5 represents the transection about the middle region of the optic lobes. The optic tecta are prominently developed. Fishes with atrophied eyes such as *Trypanchen vagina* and *Amphipnous euchaia* have been noticed

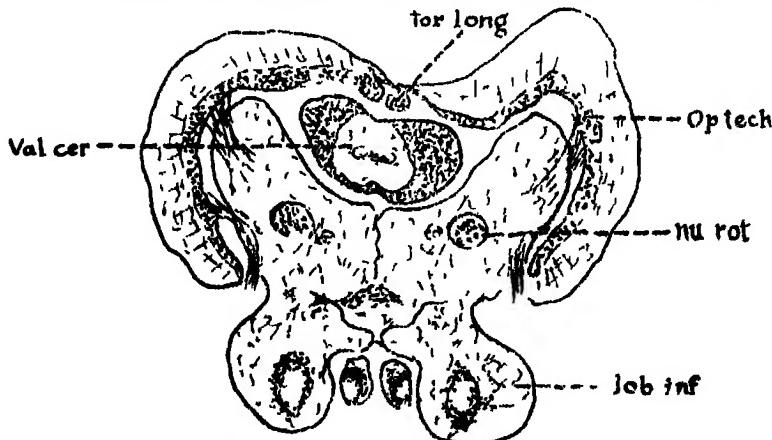


FIG. 5 Cross-section of the mid-brain of *Mugil corsula* in the region of the valvula cerebelli. $\times 30$

to possess relatively small optic tecta. The usual Teleostean mesencephalic nuclei and nerve fibres are noticed in the Mullet. There is a well-developed valvula cerebelli. The torus longitudinalis is remarkably well developed.

The rostral end of torus longitudinalis (Fig. 6) which joins the commissura posterior is particularly thick. This structure is also small in blind fishes (Kappers and others, 1936)

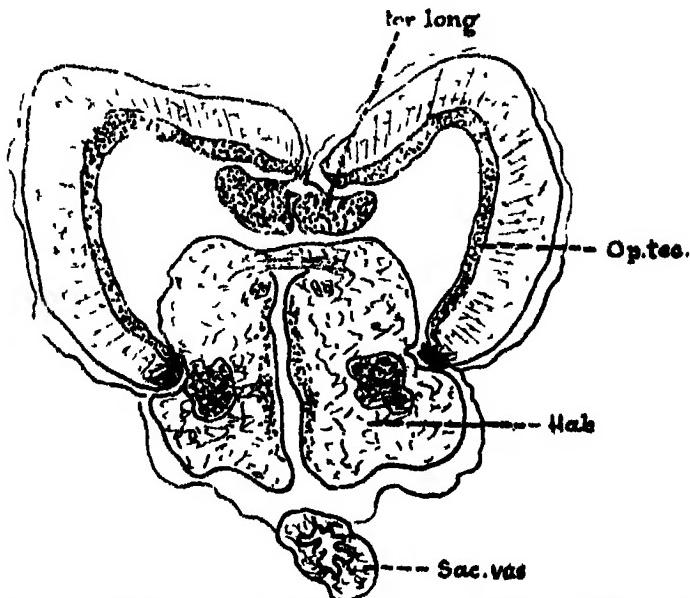
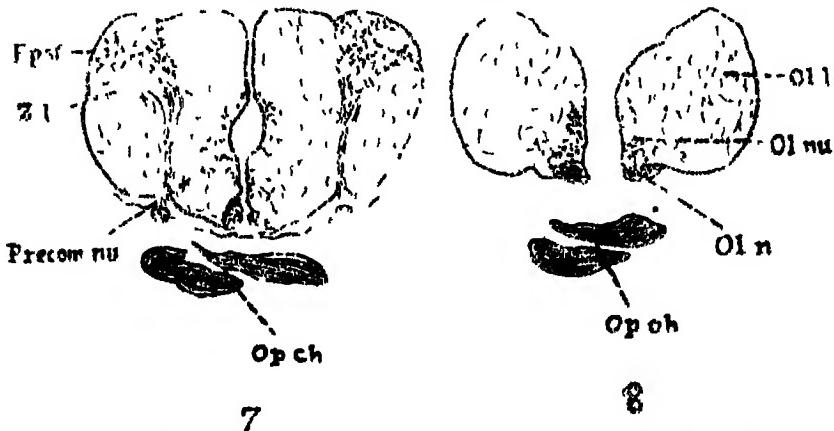


FIG 6 Cross-section of the rostral part of the mid-brain of *Mugil cornuta*
showing prominent torus longitudinalis $\times 30$

The telencephalon or the forebrain is seen in Fig. 7. The epistriatum or the primordial hippocampi is well developed. The fibre connections of this



Figs 7-8—Fig. 7 Cross-section of the fore-brain of *Mugil cornuta* showing well-developed epistriatum $\times 10$ Fig. 8 Cross-section through the olfactory lobes of *Mugil cornuta*. $\times 30$

important structure could not be investigated as the material fixed in Bouin's fluid was not well suited for the study of fibre tracts. A few sections rostrally is seen the transection of the olfactory bulb as in Fig. 8.

DISCUSSION

As the Mullet has acquired a surface feeding habit it feeds largely by sight and therefore the gustatory organs, namely the tastebuds in the mouth and on the body, particularly abundantly found on barbels of fishes which grub and grope for food at the ground level, are practically absent. Correlated with the hypertrophy of the gustatory mechanism, the vagal and the facial lobes which are the brain terminals for the nerve fibres of this system are feebly developed. These lobes are remarkably well developed in carps and catfishes which possess a highly organised gustatory system. The gustatory tracts are also poorly developed in the Mullet. The general tactile sense is well organised and as such there is a well-developed somatic sensory or the V lobe.

The sense of hearing is more acute in the surface living fishes as they are constantly exposed to the external atmosphere, than in the case of those which habitually live at the bottom (Bhimachar, 1935). Correlated with the better development of hearing the central acoustic lobe is well developed in the brain of the Mullet.

It is clear from Dr Hora's account (1938) of the habits of the grey mullet that it is a very active fish. Correlated with the active habits of the fish the cerebellum is well developed as the cerebellum is intimately connected with all the sensory centres which are concerned with the adjustment of the body in space and motor control in general. The maintenance of the muscular tone and of bodily posture are the most important functions of the cerebellum (Herrick, 1931).

As a result of the remarkable development of the visual apparatus which is adapted for aerial vision also, the optic tectum and the associated structures in the midbrain are very well developed.

The presence of a well-developed epistriatum (Fig. 7) in the forebrain is significant. There has been a great confusion regarding the exact function of the epistriatum. It is generally agreed by majority of workers that this structure in fishes has gradually lost its primary olfactory function and has become differentiated into a higher correlation centre, more or less representing a structure related to the pallium or cortex of higher vertebrates. It is to signify the higher co-ordinating nature of this structure that it is termed epistriatum by Kappers, primordium hippocampi by Johnston, paleostriatum

by Sheldon and primordium pallii by Holmgren (Kappers, Huber and Crosby, 1936) But still fishes are regarded as devoid of intelligence They are even termed "reflex machines" (Norman, 1931) Whether to treat the epistriatum of fishes as a true cortical centre of their brains is still a matter of controversy It may be pointed out that many of the Indian fishes, especially those with partial terrestrial habit as in the case of air-breathing fishes, offer excellent material for solving this problem

It is only in recent years that it is definitely established that fishes do "hear" Just as the sense of hearing was denied to fishes for a long time because they did not possess a counterpart of the cochlea of higher vertebrates, it is equally erroneous to deny them "intelligence" because there is not a true cortex in their brain

It must be admitted that for an active fish like the Mullet, a surface feeder which thrusts its head outside water for considerable periods, the visual and the auditory senses are of vital importance both from the point of view of feeding and defence With the greater development of these organs there should necessarily be higher co-ordination with various centres in the brain Dr Hora has pointed out that while chasing caddis flies the grey mullet makes a determined effort to catch them Such an action cannot be dismissed as a stereotyped reflex action It certainly constitutes intelligent behaviour

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ABBREVIATIONS

<i>ac tub</i>	Acoustico lateral tubercle	<i>nu rot</i>	Nucleus rotundus
<i>Cen.ac l</i>	Central acoustic lobe	<i>ol blb</i>	Olfactory bulb
<i>Cer</i>	Cerebellum	<i>ol n</i>	Olfactory nerve
<i>Com.inf</i>	Commissura infima	<i>ol nu</i>	Olfactory nucleus
<i>Com post</i>	Commissura posterior	<i>op ch</i>	Optic chiasma
<i>Cr cer</i>	Crista cerebelli	<i>op l</i>	Optic lobe
<i>Dec f</i>	Decussating fibres	<i>op tec</i>	Optic tectum
<i>Epist</i>	Epistriatum	<i>precom nu</i>	Precommissural nucleus
<i>Fac l</i>	Facial lobe	<i>Sec Vas</i>	Saccus Vasculosus
<i>f/b</i>	Fore-brain	<i>Tor long</i>	Torus longitudinalis
<i>F long med</i>	Fasciculus longitudinalis medialis	<i>V</i>	Ventricle
<i>g l + g r</i>	Great longitudinal secondary gustatory tract	<i>Vag l</i>	Vagal lobe
<i>Hab</i>	Habenula	<i>Val cer</i>	Valvula cerebelli
<i>lob inf</i>	Lobi inferioris	<i>Z l</i>	Zona limitans
<i>m c</i>	.. Methuner's cell	<i>VII n</i>	VII nerve
<i>med ob</i>	Medulla oblongata	<i>VIII n</i>	VIII nerve
<i>nu amb</i>	Nucleus ambiguus	<i>IX n</i>	IX nerve
		<i>X n</i>	X nerve

AN INTERIM REPORT ON THE CROP-CUTTING SURVEY FOR ESTIMATING THE OUT-TURN OF WHEAT IN THE PUNJAB (1943-44)

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THE fact that yield statistics of food grains crops in India are extremely unsatisfactory, and the principle that only crop-cutting experiments carried out on randomly selected plots give reliable estimates of the average yield per acre have been recognised by those concerned with the estimation of crop yields for a long time. The first authoritative recommendation that crop-cutting experiments must be done in randomly selected villages and in randomly selected fields came from the Board of Agriculture in the year 1919. The earliest experiments based on the principle of random sampling were carried out by Hubback (later Sir John Hubback) in the years 1923-25. Hubback's methods were used by Deshmukh (now Sir C D Deshmukh) in the CP in 1928-30. But apart from these attempts, no Provincial Government with the exception of Madras appears to have taken any measures to give effect to the recommendation of the Board even though this recommendation had been repeated by the Board and other high officials since the Board's first meeting in 1919. The two probable reasons for this situation seem to be (1) unwillingness to make any serious departure from the traditional departmental routine by the introduction of innovations, and (2) unwillingness to incur permanently any heavy additional expenditure involved in augmenting existing staff which it was thought would be necessary in adopting the method of random sampling.

2 The whole question of improving the yield statistics was considered at a meeting of the Inter-Departmental Committee of the Government of India which recommended that I should prepare a scheme keeping in view that only the minimum essential changes are made in the current procedure of conducting crop-cutting experiments, and that further the technique should be so simple that it can be worked by the existing staff, who have been already conducting these experiments, without any heavy additional expenditure. Accordingly I prepared a scheme for the sample survey which was approved by the Committee at its next meeting. The original scheme will be reproduced in the fuller report. In this interim report I shall give

only a brief account of the organisation which was set up in the Punjab and the plan of work actually adopted

3 The survey was organised under the administrative control of the Director of Agriculture, assisted by his departmental staff comprising of the Assistant Director of Agriculture, the Deputy Directors of Agriculture in charge of circles, the Extra Assistant Directors of Agriculture in charge of districts and the Agricultural Assistants in charge of tehsils. A Statistician was also attached to the survey with his headquarters at Lahore for assisting the field staff in their work.

4 The survey was organised in 27 out of the 29 districts of the Province. The Lyallpur district could not be included as some special investigation connected with the technique of the survey was in progress. The hilly district of Simla was left out because the area under wheat in the district is negligible. In what follows in this report, the word 'Province' denotes the 27 districts covered by this survey.

5. The plan of sampling was based primarily on the results of previous sample survey conducted by the I C C C on cotton in the district of Akola. Briefly the conclusion reached in the Akola survey was that a plan of sampling by tehsils which includes approximately 2 per cent of the crop-growing villages in the Province, 3 fields in each village and one plot in each field is practicable and adequate to furnish (a) a reliable estimate of the average yield for the Province as a whole; and (b) reliable quinquennial estimates of 'normals' in each district.

6 A total of 748 villages, representing approximately two per cent of the total number of villages in 27 districts was selected under the plan. These were divided among the 27 districts approximately in proportion to the area under wheat in each district, but within a district the number was divided equally between the tehsils.

7. The villages within a tehsil were selected at random. The selection was made with the help of printed random numbers to ensure equal chance of inclusion for every village in the tehsil. The selection was done personally by me at the centre, since I had anticipated that considerations of distance, of lack of communications and other difficulties might weigh with the provincial staff and be allowed to interfere with the random character of the sample.

8. Within a selected village three random fields from amongst all the wheat-growing fields, pure and mixed, were chosen and within a selected field, a plot measuring 66' × 33' was located at random. The selection of fields

and plots was done by the Agricultural Assistants but was supervised by the Extra Assistant Directors of Agriculture, and the Deputy Directors of Agriculture. The selection was so made that every wheat-growing field in the selected village got an equal chance of being included in the sample. The plot in a selected field was also marked at random in accordance with the instructions drawn for the purpose. All the operations of harvesting, threshing, winnowing and weighing were carried out in the presence of the Agricultural Assistants on days previously fixed in consultation with the owners. Although the fields were selected in advance to ensure that all fields in the village got an equal chance of being sampled, the plots were marked on the dates on which the produce was harvested.

Under the scheme villages and fields with smaller area under wheat will proportionately get larger representation in the sample. This, however, is unavoidable since there is no other practical method of selecting a random sample of plots. Even if there is association between the yield and area in the village (or field), the resulting bias in yield estimate can be corrected by weighting each village (or field) by its area when calculating the average.

9 It is known that the estimate of the average yield for a tehsil attains the maximum precision when the proposed number of experiments is so distributed that one experiment each is conducted in a different village of the tehsil. A word is, therefore, necessary to explain the decision to have three experiments in a village, and one experiment in a field. There are clearly three sources of variation within a tehsil affecting its average yield (a) the variation between villages, (b) the variation between fields in a village and (c) the variation between plots in a field. Of these (a) and (b) are generally found to be appreciably larger than (c) and explain why one plot was fixed in each field. As between (a) and (b) no appreciable difference is noticed. The distribution of experiments while depending on the ratio of these variations also depends on other considerations, such as the staff available, the time required to travel from one village to another and the necessity of completing the work in the tehsil within the period available for harvesting. As only one agricultural assistant was available per tehsil and as he can, at best, be expected to visit between 6 to 8 villages during the harvesting period and as further the variability from field to field was pronounced the consideration of spreading our experiments over as large a number of villages as possible and yet of conducting all of these within the available time led us to adopt the scheme as outlined above.

10 Departmental crop-cutting experiments, everywhere in India, are done on large plots of the order of 1/10th of an acre size. On the other

hand previous sample surveys in India, in England and U.S.A. have been mostly conducted, on small size plots. The factors which led me to the choice of large plots are.—

- (i) The coefficient of variation of plot yields decreases as the plot size increases, but within the range of large size plots the choice of a larger plot does not appreciably reduce the standard error of the estimated average yield.
- (ii) The estimated average yield attains the maximum precision when the proposed number of plots in a village is so distributed that one plot each is allotted to a different field in the village. One plot per field of large size is, therefore, statistically superior to several small size plots in a field.
- (iii) The results of the previous surveys conducted by the Indian Central Cotton Committee and by the Imperial Council of Agricultural Research show that the internal variation of a field is a very minor component of the gross variability of the final estimate and that, therefore, relative to the effect of number of experiments and their distribution, the effect of plot size on the precision of the average yield is negligible.
- (iv) The choice of large plot is in keeping with the principle that the surveys should be conducted with as little modifications in current practice as possible.
- (v) With the unevenly sown crops in India, the use of very small size plots in the hands of the existing staff who are accustomed to large size plots would lead to large errors in the location and measurement of plots. Moreover, there is a risk that considerable losses will occur in the handling of small produce and in its weigment unless arrangements are possible for processing the produce at a central place under expert supervision and with the use of sensitive balances. The large size plot is mostly free from these limitations.

Simplicity of procedure was obviously important in recommending the use of the sample survey technique by departmental agencies. The necessity of accommodating the plot within the fields without having to reject too many fields and the necessity of completing harvesting and the connected operations during the course of the day lest operations may remain incomplete and the produce may have to be left over-night without adequate supervision suggested that the plot size of 1/20th of an acre measuring 66' x 33,' would be suitable pending further investigations on the point.

There is an inherent difficulty in sampling from a finite field to which my attention has been drawn by Fisher. Whatever be the size of plot, the central portion of the field is relatively over-sampled as compared with the wide surrounding areas. The bias in sampling becomes smaller with smaller size plot, but is not completely removed. The remedy lies in introducing a correction factor such that it would equalize the chance for every portion of the field being included in the plot. If p is the probability that a sample plot occupies a particular portion of the field and q is the probability of equal chance for all portions of the field, then the proper allowance for the inequality is the weighted average of plot yields in which the plot yield has a weight q/p . The application of this correction factor to the plot yields in the present scheme shows that the estimated average yield remains unaltered, the difference between the original estimate and the corrected one being less than half of 1 per cent.

11. A detailed set of instructions for the conduct of the proposed field work, namely (a) selection of fields, (b) location of plots, (c) harvesting and the connected operations, and (d) driage was prepared for the guidance of the staff. These will be given in the fuller report. Four forms of returns were prescribed under the instructions. In form 1 of returns, the Agricultural Assistant with the assistance of the Patwari concerned is required to show the Khasra numbers of all the wheat-growing fields in the selected village, arrange these serially and to select three fields out of these by using the random numbers supplied to him. In form 2 of the returns he is required to give, in respect of the three selected fields, the pairs of random numbers selected for locating plots, the type and level of the soil, preceding crop, preceding manure, manuring in current season, whether the field was irrigated or rain-fed, the variety of wheat grown, name of the crop mixed with wheat if any, proportion of wheat in mixture, estimated yield of wheat per acre and finally the date fixed for harvesting. The Agricultural Assistant was also asked to give detailed remarks in regard to the general condition of the crop. Both the forms 1 and 2 were required to be sent to me immediately after the work of selection was over. Under form 3 of the returns the Agricultural Assistant is required to fill in the actual results of harvesting in respect of each plot in a village. The Agricultural Assistant was specially asked to note any changes in the condition of the crop that might have taken place since the fields were selected. The return was required to be sent immediately after the harvesting in each village was over. Form 4 sets out the results of driage.

12. In a scheme of this nature the entire success of the survey depends on the reliability of field work, and, therefore, on the thorough

training of the staff, and consequently the greatest attention was paid to this aspect of the work in this scheme. The training of the senior staff was commenced on the 12th March at Lahore. All the Deputy Directors of Agriculture and the Extra Assistant Directors of Agriculture as also the Provincial Statistician and the Statistician attached to the scheme were present. The plan of work, the duties and responsibilities of each member of the field staff, the meaning and implications of the instructions drawn for the actual conduct of the field work, the manner in which the returns were to be filled and despatched, were all explained in detail to the staff. They were also told how to draw up the programme of work of the Agricultural Assistants under them and how to supervise and check the conduct of their work. The training was completed with a practical demonstration of the actual conduct of field work arranged in villages around Lahore. Before they dispersed, each of the Deputy Directors of Agriculture and the Extra Assistant Directors of Agriculture was supplied with the requisite number of copies of instructions for field work, copies of the various returns and the sets of random numbers and all the equipment required for carrying out the field work.

13. Immediately on arrival at their respective headquarters, the Deputy Directors of Agriculture in turn commenced the training of the Agricultural Assistants under them. In addition to the practical demonstration of field work in neighbouring villages, the junior staff were also assigned independent villages in the neighbourhood to test whether they had understood the work. In addition to these arrangements, the Statistician attached to the scheme and the Provincial Statistician of the Department of Agriculture went round from district to district training the field staff in the conduct of field work. Altogether it took about a month to complete the training and arrangements of work.

14. After receiving training the Agricultural Assistants went back to their respective tehsils and proceeded to locate villages selected for crop-cutting work. They did not have much difficulty in locating villages since they had already worked in the tehsils and had with them maps showing the detailed lay-out of their tehsils. After drawing up the programme of their work in consultation with the Extra Assistant Directors of Agriculture, the Agricultural Assistants visited the villages, one by one, selected three fields in each village as laid down in the instructions, and in consultation with the owners of the fields fixed suitable dates for harvesting. On the days appointed they went to the village concerned with their mukkadams and got the plots harvested, threshed, winnowed and weighed in their presence.

15. The list of fields growing wheat, pure or mixed with other crops, was copied from the records of the patwari. In nine out of the selected villages the *girdawari* was incomplete and so no experiment could be conducted in them. The fields were selected with the help of random numbers supplied. The plots were marked with the help of another set of random numbers supplied for the purpose. The whole procedure of selecting the fields and plots by means of random numbers was so arranged that it could be checked by examining returns at the centre.

16. Excepting in rare cases, cultivators of the selected fields readily agreed to have the experiments done in their fields and gave their whole-hearted co-operation. In most cases they arranged for the labour required for harvesting, and in return were paid a fixed amount towards labour charges. The Deputy Directors of Agriculture and the Extra Assistant Directors of Agriculture supervised the work and satisfied themselves that the fields and the plots were selected in accordance with the procedure laid down. As a rule, wherever possible, the operations of harvesting, threshing, winnowing and weighing were done on the same day, but in cases where the produce was moist, it was allowed to dry up under the care of the Assistant and the mukkadam and was threshed after a day or two in their presence. The produce obtained was invariably weighed with the help of weights which were standardised.

17. The returns filled in by the Agricultural Assistants began to reach me from the middle of April and continued to come in till about the beginning of June. The returns which were over 3,000 in number, were scrutinised in the office, as they came in. Our scrutiny showed that the information supplied, including that on the general condition of the crop at the time of selection and on the changes in the condition of the crop since the date of selection was detailed and complete. The information provided a good means of detecting dishonest work and gave a ready explanation wherever eye-estimates and the actual yields differed a good deal. Thus rains and hailstorms had damaged the crop during the interval between the date of selection and of harvesting, occurrence of disease like rust and blight during the interval was also reported from some places. In a few cases the grain was found to have developed during the interval resulting in larger yields than those expected at the time of selection. The position of the plot itself gave an explanation for the difference in the estimated average yield for the field and the actual harvested yield. Thus plots often lay in a portion which was either better or poorer in comparison with the average, often there were *kalar* patches in the plots, often they lay by the

roadside, were covered by shade of trees and so on. Often there was a path, a nala or a hill torrent going across the plot resulting in smaller yield per acre than would be available had the whole of the area been under wheat.

18 Out of a total of 2,208 experiments conducted under the survey, a small number had to be rejected on account of incomplete information. Thus from one village it was reported that adequate labour being not available, threshing and weighment could not be completed by evening. In another distant village it was dangerous to continue threshing and winnowing after 6 o'clock for fear of thieves and animals from the adjoining forest, and, therefore, the operations had to be left incomplete. In a third village the produce was tied up and left over, but was found to be stolen the next day. In a few cases the plots lay in fields which were subsequently described as *kharaba*, and were specifically reported to be excluded from the area under the wheat crop. In other cases, the crop, being green, could not be threshed on the same day, it was left over for a day or two to dry up, but the Agricultural Assistant having found it impossible to supervise the operations on the succeeding days, the returns were excluded from the analysis. In a few cases the cultivators harvested the selected fields before the dates fixed for harvesting. In one village standard weights were not used for weighing the produce. Altogether 59 experiments were rejected on account of incomplete information. Data for the remaining experiments have formed the basis of our estimates and analysis.

19 The survey which is admittedly in the nature of experiment in method was conducted with a fourfold object; (a) to determine the average yield of wheat per acre and the total out-turn for the province with their sampling errors, (b) to collect ancillary information in respect of the acreage under different factors such as varieties, irrigation, manures, soil, previous crops and others, (c) to study the variability with a view to test the efficiency of the sample technique employed in the survey and to suggest improvements for reducing the sampling error in future years, and (d) to test the possibility of an alternative approach of eye-estimation on random fields for estimating the yield.

20 As all the data were received only a month ago, it has not been possible to undertake the study of items (b), (c) and (d). All that has been possible to do is to work out the average yield per acre, the total out-turn and their sampling errors. These are described in the following sections.

21. Table I shows the classification of selected fields as irrigated and rainfed, and as pure and mixed.

TABLE I
Number of Plots

	Irrigated	Rainfed	Total
Pure	998	591	1589
Mixed	245	315	560
Total	1243	906	2149

It will be seen from the table that 58 per cent of the selected fields are irrigated and the remainder rainfed. These percentages are in good agreement with the official figures for the percentage of area under irrigated and rainfed wheat, viz., 57.43, and demonstrate the representative character of sampling. The table also shows that roughly 20 per cent of the fields under irrigated wheat and 35 per cent. of the fields under rainfed wheat are sown mixed with other crops mostly gram. The corresponding official figures for acreage are, however, not available, as no separate record is maintained of the area under pure wheat and of the area sown with wheat mixed with other crops. What is available is the total of the acreage under pure wheat and the area under wheat as separated by the village accountant from the mixed fields. In the official procedure of calculating the out-turn the whole of this area is considered as area under pure wheat and is multiplied by the figure for the average yield per acre as found from crop-cutting experiments on only pure wheat-growing fields. The calculation involves an assumption that wheat gives the same out-turn per acre whether it is grown pure or mixed with other crops.

22 In Table II are set out the calculated averages for the yield of wheat in maunds per acre from the returns of actual yields of wheat reported to us

TABLE II
Average yield of Wheat in Maunds per Acre

	Irrigated	Rainfed
Pure	12.3288	7.4886
Mixed	8.7508	5.6420

The first row of the table gives the average yield of wheat in maunds per acre for fields sown solely with wheat, while the second row gives the average yield of wheat only in maunds per acre from fields sown with wheat in mixture with other crops. The latter are naturally less than the former,

as wheat occupied only a portion of the area in mixed fields. The appropriate method of estimating the total out-turn is to multiply these several averages by the corresponding areas. These are, however, not available, and we have to look for other methods of utilising the data for estimating the total out-turn.

23 One alternative is to multiply the official figure for the area under wheat by the average yield of wheat calculated from experiments on pure wheat fields only. This method of calculating the out-turn is, in fact, the official procedure, but as has been pointed out above, it involves the assumption that wheat gives the same out-turn per acre whether grown pure or mixed. Moreover, from the statistical point of view we cannot justify the throwing away of this relevant information in respect of experiments on mixed fields when we have already spent time, labour and money on obtaining it. On the other hand, the limitations of official acreage statistics are a handicap in the way of utilising this information. Having foreseen this difficulty we collected information on the estimates of the proportion of area under wheat in mixed fields selected for the survey. This information is made use of in estimating the average yield of wheat per acre by dividing the actual harvested yield of wheat by the proportion of the area under wheat. Table II modified in this light is set out as Table III.

TABLE III

Average Yield of Wheat in Maunds per Acre

(The appropriate standard errors are given in brackets just below the corresponding averages)

	Irrigated	Rainfed	Total
Pure	12.3238 (0.1858)	7.4886 (0.2007)	10.5254 (0.1385)
Mixed	12.2284 (0.3627)	8.5576 (0.3099)	10.1627 (0.2417)
Total	12.3046 (0.1572)	7.8003 (0.1696)	10.4309 (0.1202)

The marginal figures represent the average yield in maunds per acre for different heads. The marginal averages under the irrigated and the rainfed classification multiplied by the corresponding areas give the out-turn of wheat for all the 27 districts covered by the survey. These are shown in Table IV.

TABLE IV

	Irrigated	Rainfed	Total
Average yield in maunds per acre	12.3046	7.8803	
Acreage under wheat	5,218,000	4,019,100	
Out turn in tons	2,375,550	1,145,930	
Official estimate in tons	2,319,000	961,000	3,280,000

In the last row of the table is shown the official estimate for the out-turn of wheat in tons for the 27 districts. It will be seen that the sample estimate of out-turn of irrigated wheat is in close agreement with the official figures but that of unirrigated wheat is higher by over 19 per cent than the corresponding official figure. The sample estimate of the total out-turn of wheat for the 27 districts is higher by 241,000 tons or by 7.4 per cent.

24 Whereas the estimate we have derived relates to the yield threshed the very day or the next on which it is harvested, the general practice in the province is to allow the harvested produce to dry in the field and/or on the threshing floor for about a week or two before it is actually threshed. The total out-turn is considered as that out-turn which is obtained from threshing the harvested crop in accordance with this general practice. We have, therefore, to allow for drage from our estimate. The results of reweighment of 5 seers of sample after a fortnight reported to us give an average drage of 2.0 chhatanks equivalent to 2.5 per cent. Thus we have to deduct to bring our estimate in line with the official estimate.

25 As against this some unavoidable losses have occurred during the process of harvesting, threshing and the connected operations. As the threshing had to be done on the day of harvesting to ensure the reliability of results, all the grain could only with great difficulty be separated from the ear-heads. Although every effort was made to separate all the grain, yet a little grain, particularly where the crop was moist, escaped in the ear-heads. The grain so left was excluded from the weighment. There also appears to be a long-established practice of leaving a few ear-heads in the fields for the menials of the village. Despite our instruction that every ear-head should be collected from the experimental plots, some losses have occurred on this score here and there. There is no means of estimating the loss on these heads, but we believe it was negligible. Assuming it to be one half of 1 per cent we shall deduct 2 per cent from our estimated out-turn and obtain the net estimate of 2,328,000 tons of irrigated wheat and 1,123,000 tons of unirrigated wheat as against the official figures of 2,319,000 and

961,000 tons respectively It will be seen that the Punjab have under-estimated their production of unirrigated wheat, the increase revealed being 162,000 tons The total net increase in out-turn of wheat is 171,000 tons.

26 It has, however, to be remembered that our estimate is subject to sampling error, which is an index of the accuracy of the estimate It shows the extent to which the sample estimate is likely to differ from the actual unknown value It arises from the fact that whereas the total out-turn is the out-turn from all fields, the sample estimate of the out-turn is derived from only a few fields It is beyond the scope of this report to discuss the method of calculating the sampling error It will suffice to say that its magnitude works out to be a little over 1 per cent It shows that it is extremely unlikely that the actual out-turn is outside the margin of error of two per cent on either side of our estimated out-turn In other words, we can be almost cent per cent sure that the Punjab has not grown wheat less than 3,379,700 tons or more than 3,517,700 tons Considering that only 108 acres out of a total of nine million acres were sampled to derive the estimate, it is indeed remarkable that we should have obtained an estimate with such a low margin of error

27 One of the objects of conducting the survey was to examine the possibility of an alternative approach of eye-estimation on random fields for estimating the average yield per acre Form 2 of our return provides for information on the eye estimates of the yield of wheat per acre for the fields selected for our survey. The estimates were formed by the agricultural assistants at the time of selecting the fields Table V shows the average yield in maunds per acre calculated from these estimates for pure wheat fields along with the corresponding official figures and the averages derived from the actual results of crop-cutting experiments.

TABLE V

	Irrigated pure	Rain-fed pure
Average eye estimate of out turn in maunds per acre	11.8484	8.5756
Sample estimate in maunds per acre	12.0773	7.3388
Official estimate in maunds per acre	12.0986	6.5086

It will be seen that there is a close agreement between the three sets of figures for irrigated wheat For rain-fed wheat the eye-estimate and the official estimate closely agree, the sample estimate is however higher The Punjab is the only province in India where the average yield per acre is directly estimated in terms of maunds per acre In other provinces the estimate

is obtained in a round-about way by multiplying the so-called 'normal' with the condition factor of that year. We are reporting elsewhere the comparison of the results of the method of anna estimate with the actuals. Here it will suffice to state that 'anna' system is understood differently by different men, and leads to errors of very large magnitude. We have not had time to analyse the data for reporting on the personal bias of different workers in forming the eye estimates nor we have had time to work out the correlation between the eye estimate and the actual out-turn. The results reported above, however, show the possibility that if eye estimates by the agricultural staff are obtained on a large number of fields selected at random, it should be possible to estimate the actual out-turn with considerable precision.

28 The net increase of 5.2 per cent revealed by the survey is small, but the increase of 17 per cent in the out-turn of unirrigated wheat is rather large. In anticipating difference between the sample and the official estimates it has to be remembered that an experienced administrator can derive a fair idea of the average yield per acre for his district by conducting a few crop-cutting experiments even in accordance with the existing defective procedure. In the very nature of things, therefore, it is unlikely that his estimate can be wide of the mark by more than 15 to 20 per cent. margin of error. In a vast country like India, moreover, even a small percentage of difference means hundreds of thousands tons of production. It is, therefore, necessary to estimate the actual production with the maximum precision. The real advantage of conducting crop-cutting surveys in accordance with the procedure outlined in this report is that it achieves this object. There is another aspect which brings out the utility of the crop-cutting survey in the estimation of out-turn. There is a tendency in official estimates to keep close to the average of preceding years. The result is that the true fluctuations in the out-turn from year to year are not brought out. These fluctuations may be important in determining the policy and can be accurately assessed only by means of conducting crop-cutting surveys. The results of future surveys alone will show the advantage of crop-cutting surveys. In the meantime it is necessary to repeat the survey for perfecting the technique and for examining the possibility of an alternative and cheaper approach of forecasting yields. Such a survey incidentally will give us a picture of the agricultural conditions prevailing in different tracts of the country and over a period of years reliable estimates of normal yields for each district.

29. This is the first large-scale survey of its type carried out over an area of 88,000 square miles. The success of it is due to the whole-hearted co-operation of the Department of Agriculture in the Punjab. They can well be proud of their work, particularly as they will be giving a lead to other provinces in maintaining accurate statistics for their out-turns. The encouragement and the keen interest of the senior officials of the Government of India at the various stages of this survey contributed in no small measure to its success. Particular mention must be made of the help received from Sir P. M. Kharegat, Additional Secretary, Education, Health and Lands Department, Mr B. Sahay, Deputy Secretary, Education, Health and Lands Department, Mr D. R. Sethi, Director of Agricultural Production, Mr H. R. Stewart, Vice-Chairman, Imperial Council of Agricultural Research, and Mr S. M. Srivastava, Secretary, Imperial Council of Agricultural Research. It is hoped that the survey will be conducted year after year as recommended by the Inter-Departmental Committee on Agricultural Statistics, and would be carried out in the same spirit and with even greater efficiency than has been done during this year. The importance of the results of such surveys far outweighs the expense involved, which in itself happens to be only a little more than a thousand of rupees per district.

ON THE PRESENT STATUS OF THE GENUS *LOXOGENES*

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THE writer (1943) described two new trematodes from the intestine of frogs and toads from Kashmir, and, reviewed the genus *Pleurogenes*. In the course of the discussion it was pointed out that *Loxogenes arcum* (Nickerson, 1900) Stafford, 1904, and *Loxogenes bicolor* Krull, 1933, cannot be placed in one and the same genus *L. arcum*, the type species of the genus *Loxogenes* was shown to resemble the species of the genus *Pleurogenoides*, and was consequently removed and put under the latter genus (*Pleurogenoides*), leaving *L. bicolor* as a distinct form. Under the International Rules of Zoological Nomenclature, a new generic name is, therefore, necessary for the reception of *L. bicolor* Krull, 1933. The genus *Loxogenoides* (n.g.) is, therefore, created for its reception, with the following diagnosis —

Loxogenoides n.g.

Medium sized distomes with broad, flattened and thick body; cuticle with spines, oral sucker well developed and subterminal, acetabulum small and pre-equatorial, prepharynx absent, pharynx present, oesophagus short and intestinal cæca long and inflated, reaching near the posterior end of body, testes large, elongated dorsoventrally and folded, post-equatorial and connubial, cirrus sac long and slender, pre-equatorial and dextral; ovary round, lobed and pre-testicular, lying dorsal to acetabulum, receptaculum seminis and Laurer's canal present, shell-glands well developed, vitelline follicles pre-acetabular with greatest concentration laterally, uterus voluminous, both pre- and post-acetabular, metraterm short and well differentiated, genital pore ventral, pre-acetabular, and dextral; excretory vesicle Y-shaped with a long stem and short cornua; excretory pore terminal; parasites of Amphibia.

Type species.—*Loxogenoides bicolor* (Krull, 1933) (syn. *Loxogenes bicolor* Krull, 1933)

Corrigenda to the "Studies on the Helminth Parasites of Kashmir,
Part II," by B. L. Kaw

Page 100, line 34, Read *bufonis* for *Bufo*

Page 103, line 1, Read *P. japonicus* for *P. japanicus*

" line 38, Read *P. japonicus* for *P. japanicus*

Page 105, lines 38-40, Delete "Thus the author . . . representative"

Page 106, line 1 Read *Loxogenoides* (n.g.) for *Loxogenes* Krull (1933)
Emended

" line 2, Read diagnosis for diagnoses

" line 16 Read *Loxogenoides bicolor* for *Loxogenes bicolor*.

Page 107, line 5, Read *lobatus* for *Lobatus*

" lines 15-16, Read *P. arcanum* (Nickerson, 1900) Stafford, 1904, for
P. arcanum Klein, 1905.

" line 18, Read *japonicus* for *japanicus*

" line 19, Read *P. bufonis* for *P. bufo*

" line 20, Read *Loxogenoides* (n.g.) for *Loxogenes* Krull (1933)

REFERENCE

Kaw, B. L.

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1943

PYHSIOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY SAPROLEGNIACEÆ

Part II. Sulphur and Phosphorus Requirements*

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(Communicated by Prof B C Mahendra, F Z S, F A S C)

INTRODUCTION

THE importance of the knowledge of the nutritive relations of a fungus for cultural purposes is well known. The foremost consideration which forms a part of the general programme for its study is the formulation of a synthetic nutrient medium of known definite composition suitable for its growth.

A review of the available literature shows that adequate attention has not been paid to the study of sulphur requirements of the fungi. The author is of opinion that sulphur plays a very important rôle in their nutrition. An unsuitable sulphur compound in the nutrient medium may lead to erroneous results. Leonian and Lilly (1938), for example, while studying the effect of various nitrogenous substances on the growth of *Achlya conspicua*, *Aphanomyces campylostylus*, *Isoachlya monilifera*, *Saprolegnia mixta* and *S. parasitica* among others, used a nutrient medium containing KH_2PO_4 , MgSO_4 , NH_4NO_3 , dextrose and distilled water. The fungi named above failed to respond to the above medium even in the presence of thiamin, but showed growth in the presence of an aminoacid, viz., 1-cystin. From this they concluded that this aminoacid and not NH_4NO_3 was the proper source of nitrogen. The work of several investigators (Volkonsky, 1933, 1934, Dayal, 1942, and Bose, 1943) has amply proved that Saprolegniaceous fungi are unable to utilise sulphates but readily take up cystin and sulphides. It appears to the author that in the above case the nutrient medium lacked in a proper source of sulphur which was accidentally supplied by 1-cystin, when added as a substitute for ammonium nitrate.

Fungi have been cultured on a large number of media containing various sulphur compounds. Recently Steinberg (1941) from his extensive study of sulphur nutrition of *Aspergillus ruger* was able to conclude that sulphur was reduced to sulphoxylate prior to its conversion to organic sulphur.

* Part of thesis approved for the D Phil Degree in the Allahabad University

and that this organism was unable to utilise sulphides and disulphides. Other biological data obtained by various workers show that fungi have been successfully grown on various sulphur compounds (Armstrong, 1921; Kossowicz and Loew, 1912, Volkonsky, 1933, 1934) including sulphides and disulphides. Fischer (Lwoff, 1932) has classified the organisms into two categories (1) "Euthiotrophe" which can obtain their sulphur from " SO_4^- " ions and (2) "Parathiotrophe" which cannot utilise " SO_4^- " ions. The present investigation was, therefore, undertaken to study the relation between constitution and assimilation of sulphur compounds in the case of *Achlya* sp., *Brevilegnia gracilis* v. Eek., *Isoachlya anisospora* (DeBary) Coker, var. *indica* Sak et Bhar, *Saprolegnia delica* Coker and *Saprolegnia monoica* Pringsh.

Along with the investigation of sulphur requirements, experiments dealing with the utilisation of phosphorus compounds were also performed.

METHODS

The fungi were grown either in culture tubes ($1.5 \times 15\text{ cm}$) or in 150 ml. Erlenmeyer flasks containing 10 and 25 c.c. of a nutrient medium respectively. The basal medium, which will hitherto be referred to as medium A, in the case of experiments dealing with sulphur requirements consisted of 0.5 gm each of KH_2PO_4 , $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 2 gm of NH_4NO_3 , 5 gm of dextrose and 1000 c.c. of double-distilled water. Various sulphur compounds were added singly to the basal medium so as to furnish 25 mg. of sulphur per litre. In the case of hydrogen sulphide, the gas was passed for 5 minutes through 200 c.c. of the basal medium. The composition of the basal medium in the case of experiments dealing with phosphorus requirements was as follows— $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ —0.5 gm., NH_4NO_3 —2.0 gm., dextrose—5 gm., Na_2S —0.15 gm. and double distilled water—1000 c.c. Sulphur supplied in the case of *Brevilegnia gracilis* was in the form of K_2SO_4 (0.5 gm. per lit.) since it supports better growth of the organism. The amount of various phosphorous compounds added to the basal medium was such as to furnish 115 mg. of phosphorous per lit.

The solutions were sterilised in an autoclave at 15 pounds pressure for 15 minutes. The hydrogen-ion concentration of the nutrient media was adjusted with NaOH and HCl so that it was approximately 7 after autoclaving. Three or more replicates were used in each of the several experiments, all of which were carried on at 25°C . The incubation period varied from 10–21 days. Only guaranteed reagents (either of Mercks or British Drug House) were used.

The stock cultures were maintained on the basal medium solidified with Difco bacto-agar. Material for inoculum was taken from the margin

of a young colony taking care that the size of the inoculum was equal (4 mm.) in all cases. For transfers from a tube to a flask containing the same medium, some mycelium was dissected out inside the tube with the help of a platinum needle and transferred aseptically. A particular substance was thought suitable only when the fungus grew on it by subsequent transfers.

Only pure cultures were employed throughout all the experiments reported below. Whenever a culture showed bacterial contamination, it was discarded.

Dry weights were determined by washing the mycelium thoroughly with distilled water on a weighed filter-paper and drying it in an electric oven at 60° C for 72 hours. The dried mats were removed from the oven to the desiccator, cooled and weighed rapidly on an analytical balance to constant weight. Since there was not much difference in the weights of colonies produced in replicate cultures, only the average values have been tabulated.

Only Pyrex glassware, thoroughly cleaned with chromic-sulphuric acid mixture and washed twice with distilled water, was used throughout.

EXPERIMENTAL

Sulphur Requirements

Medium A with and without the addition of various sulphur compounds given in Table I was inoculated with the fungi. The results are tabulated in Table I.

TABLE I
*Dry weight (in mg) of the fungal colonies grown on medium A
 with and without sulphur compounds*
(Time of incubation = 21 days)

Compounds	<i>Achlya</i> sp	<i>B. gracilis</i>	<i>I. annospora</i> var <i>indica</i>	<i>S. delta</i>	<i>S. menierea</i>
Potassium sulphate		22.3			
Sodium bisulphide		20.0	.		
Sodium sulphite		16.3			
Sodium hyposulphite	4.0	23.3	10.6	8.2	13.3
Sodium sulphide	10.0	20.3	13.7	10.0	17.3
Hydrogen sulphide	4.0	20.0	6.8	9.0	10.0
Potassium persulphate		17.3			
Sodium thiosulphate	2.0	20.3	8.0	9.8	15.7
Sodium dithionite		2.8			
Cystin	11.0	15.6	11.0	12.3	28.7
Cysteine hydrochloride	4.3	8.3	6.0	9.7	17.5
Thiourea	5.7	6.6	7.6	1.8	5.0
Medium A (control)	..	2.6		.	

It is seen from the results summarised in Table I that *Achlya sp.*, *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S monoica* did not grow on medium A and were unable to utilise sulphur in the form of potassium sulphate, sodium bisulphite, sodium sulphite, potassium persulphate and sodium dithionate. Of the inorganic compounds tried, sodium sulphide supported the maximum growth, while cystin served the best source among the organic compounds. *Brevilegnia gracilis* showed good growth in all cases except on medium A and the medium containing sodium dithionate traces of growth were present on these two. The dry weights of mycelium varied according to the sulphur compounds in the following diminishing order—hydrogen sulphide, sodium hyposulphite, potassium sulphate, sodium thiosulphate, sodium sulphide, sodium bisulphite, potassium persulphate and sodium sulphite.

Phosphorus Requirements

The basal medium with and without the addition of various phosphorus compounds given in Table II were inoculated with the fungi. The results are summarised in Table II.

TABLE II

Dry weight (in mg) of the fungal colonies grown on basal medium with and without phosphorous compounds

(Time of incubation - 21 days)

Compounds	<i>Achlya sp.</i>	<i>B. gracilis</i>	<i>I. anisospora</i> var. <i>indica</i>	<i>S. delica</i>	<i>S. monoica</i>
Potassium dihydrogen phosphate	5.0	36.7	16.0	10.0	13.3
Casein	55.0	68.3	63.3	51.0	70.0
Nucleic acid	11.0	42.0	20.0	17.0	19.0
Basal medium (control)		traces			

It is evident from the data obtained in Table II that the fungi did not grow on the basal medium lacking in phosphorous compounds, but were able to utilise phosphorus both from inorganic and organic sources.

DISCUSSION

That sulphur is essential for the growth of the organisms is shown by the absence of growth on medium A. The slight growth of *Brevilegnia gracilis* obtained on medium A, and on medium A containing sodium dithionate is due to the traces of sulphur (0.02%) present as impurities in the form of "sulphates" (SO_4) in both $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ and NH_4NO_3 (*Proanalysis* of Merck). It seems that even this small amount is sufficient for the growth.

of *B. gracilis* which is able to assimilate sulphur in the form of sulphates. Results obtained for *Achlya* sp., *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* are in general agreement with those obtained by Volkonsky (1934) for some members of the family Saprolegniaceæ. He found that they were unable to use sulphates but utilised thiosulphates, sulphhydryl and sulphides. The results of Leonian and Lilly (1938) indicate that organic sulphur is necessary for the growth of *Saprolegnia mixta*, *S. parasitica*, *Achlya conspicua*, *Isoachlya monilifera* and *Aphanomyces camptostylus* (Steinberg, 1939, p. 335). Schade (1940) found that *Leptotomitus lacteus* and *Apodachlya brachynema*, sewage water-molds, were able to reduce sulphates to satisfy their sulphur requirements. Persulphate, which served as a good source of sulphur in the case of *Brevilegnia gracilis*, proved a favourable source of sulphur for *Aspergillus niger* (Armstrong, 1921, Steinberg, 1941) and *Penicillium glaucum* (Armstrong, 1921) also. Dithionate, which is a valueless compound as a source of sulphur in the present case, behaves similarly with *Aspergillus niger*. These fungi, except *Brevilegnia gracilis*, are thus able to utilise the inorganic sulphur in a reduced form. Oxidised forms like sulphates and sulphites are not assimilated.

Phosphorus is equally important for the growth of the organisms as is clear from the results summarised in Table II. The fungi do not grow on a basal medium lacking in phosphorous compounds. No comparative value can be attached to these data since casein and nucleic acid may contain some growth stimulatory factors in addition to an extra amount of available carbon and nitrogen.

SUMMARY

Achlya sp., *Brevilegnia gracilis*, *Isoachlya anisospora* var. *indica*, *Saprolegnia delica* and *S. monoica* are unable to grow on a nutrient medium in the absence of sulphur. These fungi except *Brevilegnia gracilis* cannot obtain sulphur from sulphate, sulphite, bisulphite, persulphate and dithionate but thrive well with hydrogen sulphide, sodium sulphide, hyposulphite, thiosulphate, cystein, cystein hydrochloride and thiourea. *Brevilegnia gracilis* can utilise sulphur from all the substances tried except dithionite. All the five fungi are able to utilise both inorganic and organic phosphorous compounds, in the absence of which they show no growth.

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